

**LEVEL II**

EFFECTS OF OZONE ON EXERCISING AND SEDENTARY ADULT MEN AND  
WOMEN REPRESENTATIVE OF THE FLIGHT ATTENDANT POPULATION

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Prepared for  
U.S. DEPARTMENT OF TRANSPORTATION  
Federal Aviation Administration  
Office of Aviation Medicine  
Washington, D.C. 20591

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Technical Report Documentation Page

1. Report No. 14 FAA-AM-79-26	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle 6 EFFECTS OF OZONE ON EXERCISING AND SEDENTARY ADULT MEN AND WOMEN REPRESENTATIVE OF THE FLIGHT ATTENDANT POPULATION		5. Report Date 11 Oct 1979
7. Author E. A. Higgins, M. T. Lategola, J. M. McKenzie, C. E. Melton, J. A. Vaughan		6. Performing Organization Code
9. Performing Organization Name and Address FAA Civil Aeromedical Institute P.O. Box 25082 Oklahoma City, Oklahoma 73125		8. Performing Organization Report No.
12. Sponsoring Agency Name and Address Office of Aviation Medicine Federal Aviation Administration 800 Independence Avenue, S.W. Washington, D.C. 20591		10. Work Unit No. (TRAIS)
15. Supplementary Notes Work was done under approved Tasks AM-B-78-PHY-115 and AM-B-79-PHY-115.		11. Contract or Grant No.
6. Abstract Three studies at two ozone concentrations have been carried out in an attempt to define the effect level for ozone under simulated flight conditions. All experiments were carried out in an altitude chamber held at 6,000 feet MSL; relative humidity was kept at 10-12 percent and temperature at 68° - 74° F. Subjects were paid nonsmoking men and women in their third decade who had the anthropomorphic characteristics of airline flight attendants. All subjects were exposed to ozone in one experiment and to air only in another. Order of presentation of the experiments was balanced, and sessions were separated by 1 week. Study 1 consisted of exposure of 15 men and 12 women to 0.20 parts per million by volume (ppmv) ozone for 4 h with treadmill exercise for the last 10 min of each hour. In the second study 14 men and 14 women were exposed to 0.30 ppmv ozone for 3 h with 10 min exercise at the end of each hour. The third study consisted of exposure of 14 men and 14 women to 0.30 ppmv without exercise. Cardiopulmonary, performance, visual, and symptoms assessments were made. Hematologic and urinary studies were also made in the first two studies. In the first study no effects attributable to ozone were seen though effects of the order of presentation were seen. In the second study, all subjects reported subjective symptoms significantly related to ozone exposure, though the symptoms were not uniform throughout the group. In some subjects, symptoms outlasted the period of exposure by 6 h to 4 d. Spirometry functions were impaired ( $p \leq 0.05$ ) in males and females; no important visual effects were shown. In the third study, effects ( $p \leq 0.05$ ) of ozone were seen on forced midexpiratory flow in men only; and, for what they are worth, in photopic visual acuity and blink rate.		13. Type of Report and Period Covered 12 145
17. Key Words Air pollution, Ozone, Respiration, Vision, Blood, Heart rate, Short-term memory, Hand steadiness, Airline operations, Flight attendants, Airline passengers.	18. Distribution Statement Document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.	
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 96
		22. Price

## PREFACE

This report is the result of a good deal of coordinated effort by several investigators. In order that each investigator might have credit for his work, the report is organized into sections, each of which deals with a separate set of measurements. The Table of Contents reflects this organization. The authors of this report are listed alphabetically; this order does not imply seniority or that one author has contributed more than the others.

Page numeration runs consecutively through the report.

The authors would like to acknowledge the help of several people who contributed substantially to the prosecution of this project. Gordon Funkhouser was responsible for much of the work involved in construction and maintenance of the ozone generating and monitoring systems; he also contributed greatly to data collection and analysis. Stanley Mullen served as "inside man" in most of the experiments. Peggy Lyne and Mary Jo Burr provided excellent coverage of cardiopulmonary and symptom questionnaire measurements. Patsy Fowler was responsible for blood specimen collection and, together with Stanley Mullen and Ted Saldivar, for blood analyses. Russell Moses analyzed urine specimens for catecholamines and Patsy Fowler analyzed them for 17-ketogenic steroids. Clay Tucker took care of the standard meals served to the subjects and also many other varied tasks. Donna Fitzgerald has the thanks of the authors for typing the report.

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## SECTION I

### INTRODUCTION

C. E. Melton

#### Perspective on the Problem.

The majority of airline passenger and crew complaints attributed to ozone exposure consist primarily of respiratory tract irritation with cough and substernal discomfort. Other common symptoms are headache and/or fatigue. Symptoms are principally associated with transpolar long distance flights on Boeing 747-SP (Special Performance) aircraft. The problem is annually cyclical and is most acute in late winter or early spring in the vicinity of major atmospheric low pressure centers such as the Icelandic or Aleutian Lows (1).

It is probable in retrospect that the problem of "ozone sickness" has existed for several years, though the symptoms were attributed to other in-flight factors such as poor ventilation and low humidity. In 1973, flight attendants submitted two reports of symptoms now believed to be caused by ozone but then attributed to poor cabin ventilation. Industry responded to these and subsequent complaints with various alterations in cabin ventilation systems. In July 1976, the FAA New York International Field Office prepared a Cabin Safety Survey referencing flight attendant complaints and recommended that environmental studies be conducted. In-flight measurements of various gases, including ozone, were made by the aircraft manufacturing industry in the summer of 1976. They reported that cabin concentrations were below normally accepted industry standards. In the spring of 1977, there were many in-flight reports of the symptoms listed above. The causative agent was identified as ozone in March 1977 (i) because of the coincidence of an increase in numbers of complaints with advent of the "ozone season" and (ii) because of the demonstrated presence of ozone in cabin air (2-5).

In April 1977, the Civil Aeromedical Institute (CAMI) was requested to provide research in support of a proposed rule limiting airliner cabin ozone levels. Preparations for research were begun immediately and the first experiments were run in February 1978. The last set of experiments on flight attendant effects was completed in March 1979. This series of experiments was directed at the flight attendant population because of repeated exposure of that group and because of the physical activity involved in the performance of flight attendant duties. However, the third study in this series (sedentary conditions) is also applicable to flight deck crews and passengers. Another series of experiments is presently underway to define the effects of ozone on men (age 40-59) representative of the airline pilot population.

#### Historical Review.

The word "ozone" is derived from the Greek word "ozein," meaning "to smell." The name was given to the substance by C. F. Schönbein in 1847, who noted its peculiar odor and its effects on himself and experimental animals (6-7).

Ozone is used as an industrial bleach and disinfectant. With the advent of large numbers of gasoline-burning automobiles, ozone became a matter of general public concern because of its formation in smog by a photochemical reaction involving ultraviolet light and oxides of nitrogen. Haagen-Smit (8) in 1952 reported that oxidants, principally ozone, in Los Angeles smog were responsible for the smog's irritating properties. The report of the National Research Council (9) adequately covers the literature and summarizes the state of knowledge regarding atmospheric oxidant toxicity up to about 1975; therefore, no attempt will be made here to reproduce that excellent and comprehensive review. Reports cited in the review that are particularly pertinent to this report will be resummarized here, however.

In 1962 Bennett (5) measured ozone concentrations up to 0.065 ppmv at 41,000 ft mean sea level (MSL) in the Comet and 0.12 ppmv in the Boeing 707. In subsequent ground level experiments, Bennett showed that exposure of six men to an ozone concentration of 0.20 ppmv in an office setting 3 h/day, 6 days/week for 12 weeks was innocuous. Six other men were exposed simultaneously under the same regimen to 0.50 ppmv and complained of no symptoms but showed a highly significant downward trend in 1-second forced expiratory volume ( $FEV_1$ ) over the 12-week exposure. This finding was interpreted to mean that there was some progressive obstruction of terminal bronchioles attributable to ozone exposure. Incidence of upper respiratory infections was less in both ozone-exposed groups than in control personnel and forced vital capacity (FVC) showed no change.

Lagerwerff (10) in 1963 carried out laboratory experiments involving exposure of volunteer male and female subjects to 0.20, 0.35, and 0.50 ppmv ozone for 3 h and again for 6 h. This work showed a decrease in scotopic and mesopic vision and changes in extraocular muscle balance affecting all but the superior and inferior recti. Some of the subjects reported eye irritation that disappeared overnight, a sensation of skin tightness over the face, fatigue, and lack of ability to concentrate. Two subjects quit the project, claiming severe respiratory irritation; however, their vital capacities showed no change and other pulmonary tests were likewise reported normal.

Hore and Gibson (11) exposed 99 university students to 0.20 to 0.30 ppmv while administering intelligence tests to the students. No changes in mental ability were found.

Hackney and coworkers (12-14) described "reactive" and "nonreactive" people with respect to respiratory symptoms caused by experimental ozone exposures. Reactive people were those otherwise healthy but with a history of cough, chest discomfort, or wheezing associated with allergy or exposure to air pollution. Nonreactive individuals were not affected or only minimally affected by 4-h exposure to 0.50 ppmv of ozone. Reactive individuals showed marked changes in pulmonary function as well as

malaise, muscle ache, cough, wheezing, sputum production, substernal pain, dyspnea, fatigue, headache, laryngitis, and nasal discharge. These effects were seen during 2-h exposures at 0.37 and 0.50 ppmv while 0.25 ppmv was essentially innocuous for all subjects for 2- and 4-h exposures. Buckley et al. (15) showed significant changes in red blood cells (RBC) and sera from young men after a 2 3/4-h exposure to 0.50 ppmv ozone. RBC changes consisted of increased hemolysis by hydrogen peroxide, increased glucose-6-phosphate dehydrogenase, increased lactate dehydrogenase, decreased acetylcholine esterase, and decreased reduced glutathione. Serum vitamin E and serum lipid peroxidation levels were increased while serum glutathione reductase was decreased. The effects persisted to some extent for 2 weeks after exposure. Hackney et al. (16) also showed that reaction to ozone may be affected by previous exposure. Southern Californians, exposed more or less constantly to ozone in polluted air, were less reactive to experimental exposure to ozone (0.37 ppmv) than were Canadians who lived in relatively clean air, thus indicating that an adaptive mechanism may exist. Hackney et al. (17) further showed that five out of six reactive men experimentally exposed to 0.50 ppmv ozone adapted to the gas over 4 days to the extent that symptoms and physiological effects mostly disappeared. Linn et al. (18) showed that asthmatics experimentally exposed to 0.20 ppmv ozone showed no significant respiratory changes but did show significant increases in blood levels of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and peroxide fragility of RBCs and a decreased concentration of reduced glutathione. Linn and coworkers interpret these findings as supportive of California's smog alert level of 0.20 ppmv ozone.

Kerr et al. (19) showed that smokers were not as susceptible to ozone as nonsmokers. Ten healthy adults in each category were exposed with periodic exercise to 0.50 ppmv ozone in an environmental chamber. Nonsmokers developed more symptoms than did smokers and symptoms were correlated with decreased pulmonary function.

An extensive literature review was prepared by the American Society for Testing and Materials (ASTM) (20). In paragraph X1.2.1 of the appendix to the standard is cited literature relative to human exposure. In Table 1 of this report, the data quoted in ASTM's report are condensed to show simply the presence (+) or absence (-) of ozone effects. The table shows that there is only one report of significant effects below the level of 0.30 ppmv. This one positive effect was reported by Lagerwerff who claimed a decrease in scotopic vision, increased divergence, and increased lateral phoria caused by 0.20 ppmv for 3 h. Above 0.30 ppmv, significant effects are almost uniformly demonstrated.

Review of the literature related to experimental exposure of humans to ozone shows: (1) The biological threshold for ozone effects (aside

TABLE 1. Effect of Various Levels of Ozone in Producing Symptoms in Man

$O_3$	EFFECT
0.10	- (Pulmonary)
0.15	- (Pulmonary)
0.15	- (Blood)
0.20	- (Pulmonary)
0.20	- (Mental)
0.20	+ (Vision)
0.30	- (Mental)
0.30	- (Pulmonary)
0.30	- (Blood)
0.30	+ (Pulmonary)
0.37	+ (Pulmonary)
0.37	+ (Pulmonary)
0.37	+ (Pulmonary)
0.37	+ (Pulmonary)
0.37	+ (Pulmonary)
0.37	+ (Pulmonary)
0.40	- (Blood)
0.40	+ (Pulmonary)
0.50	+ (Pulmonary)
0.50	+ (Pulmonary)
0.50	+ (Pulmonary)
0.50	- (Pulmonary)
0.50	+ (Pulmonary)
0.50	+ (Pulmonary)
0.50	+ (Pulmonary)
0.50	+ (Blood)
0.50	+ (Blood)
0.50	- (Vision)
0.50	+ (Vision)
0.60	+ (Pulmonary)
0.60	+ (Pulmonary)
0.75	+ (Pulmonary)
0.75	+ (Pulmonary)
0.75	+ (Pulmonary)
0.75	+ (Pulmonary)
0.75	+ (Pulmonary)
0.75	+ (Pulmonary)
0.80	+ (Pulmonary)
0.80	+ (Pulmonary)
0.90	+ (Pulmonary)
1.00	+ (Pulmonary)
1.00	+ (Pulmonary)
1.50	+ (Pulmonary)
2.00	+ (Pulmonary)
3.00	+ (Pulmonary)

from olfactory effects) probably lies between 0.20 and 0.30 ppmv (400-600  $\mu\text{g}/\text{m}^3$ ). (ii) Symptoms noticeable by exposed normal people will probably first occur between 0.30 and 0.50 ppmv (600-1000  $\mu\text{g}/\text{m}^3$ ). (iii) Some people are more reactive than others to ozone exposure; reactors are found among people with asthma and allergies. It is not possible to generalize from the sparse data that exist about the lowest ozone level at which reactors will show effects. (iv) Visual effects have been demonstrated by only one set of experiments. (v) Adaptation to ozone probably occurs but the mechanism is obscure. (vi) Extrapulmonary effects (other than blood) may occur but the mechanism is unknown. (vii) The effects on humans of long-term exposure are not well defined. (viii) Effects of ozone are probably more dependent on concentration than on duration of exposure. (ix) Good evidence exists that free radical scavengers mitigate the effects of ozone. Vitamin E probably has a protective effect. Not much experimentation has been done on humans in this regard. (x) Ionizing radiation, high pressure oxygen, hydrogen peroxide, and ozone probably have similar basic actions. Radiation is somewhat more damaging because of its route of entry into the body and its deeper penetration into cells. (xi) Smokers are probably less susceptible to ozone than are nonsmokers. (xii) No report of human death from ozone exposure has been found in the literature.

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## SECTION II

### GENERAL METHODS

C. E. Melton

Eighty-three young men and women were used in three studies. The age-height-weight characteristics of the subjects and numbers of each sex are shown in Table 1. Each subject participated in two experimental episodes, one with ozone and one without ozone. All experiments were carried out in an altitude chamber at 6,000 ft MSL. Air in the chamber was dried by chilling and then rewarmed to maintain a chamber relative humidity of 10-12 percent and a temperature of 68°-74° F. Temperature/humidity data are shown in Table 2.

Ozone was generated by the action of ultraviolet light on ambient oxygen. The ozone generator is diagrammed in Figure 1. Desired ozone levels were attained with additional generators, as required, and maintained by varying the voltage with a Variac to one of them.

All subjects were paid volunteer nonsmokers, and each was given a medical examination prior to being accepted into the study. Further, all subjects were fully briefed on the purposes, procedures, and possible hazards of the study; each signed an informed consent document. Training and further screening were then carried out to insure that each subject could perform the required tests.

All subjects reported to the laboratory at 0800-0815 for experimentation. Each was given a surgical scrub suit to put on, after which he or she was given a pulmonary function test. Each subject then ate all of a standard breakfast consisting of two scrambled eggs, two slices of bacon, two pieces of buttered toast, jelly, and a glass (about 250 mL) of milk. Subjects received no water or food from then until completion of the experiment. Electrocardiographic electrodes were attached to the subject's chest. A urine specimen was collected and the subject, together with a researcher, entered the altitude chamber lock for ascent to 6,000 ft MSL. The schedule of experimentation is shown in Table 3 for the first study, in Table 4 for the second study, and Table 5 for the third study.

The first study consisted of exposure of subjects with treadmill exercise to 0.20 ppmv ozone ( $400 \mu\text{g}/\text{m}^3$ ) for 4 h, yielding a time-weighted average (TWA) of 0.10 ppmv based on an 8-h day. The second study employed essentially the same protocol as the first except that the ozone exposure level was 0.30 ppmv ( $600 \mu\text{g}/\text{m}^3$ ; TWA = 0.11 ppmv) for 3 h and the fourth exercise period was deleted. The third study was the same as the second study (0.30 ppmv ozone) except that subjects were sedentary for the entire 3-h period of exposure. Ozone levels (ppmv) are shown in Table 6.

TABLE 1. Age-Height-Weight Characteristics  
of the Subject Population

		AGE (yr)	HEIGHT (cm)	WEIGHT (kg)	N
(1) M	$\bar{X}$	23.4	179.0	72.8	15
	SE	0.7	1.5	1.6	
(2) M	$\bar{X}$	23.0	176.8	74.7	14
	SE	0.7	1.2	1.5	
(3) M	$\bar{X}$	25.2	179.2	74.0	14
	SE	1.1	1.1	2.1	
(1) F	$\bar{X}$	27.8	165.3	58.2	12
	SE	1.5	1.5	2.0	
(2) F	$\bar{X}$	26.1	166.6	62.2	14
	SE	1.3	1.1	1.5	
(3) F	$\bar{X}$	25.9	164.3	57.1	14
	SE	1.2	1.7	1.6	

M = male. F = female.  $\bar{X}$  = mean. SE = standard error of the mean.  
N = number of subjects.

Age calculated to the nearest completed year. Height was measured in  
stocking feet. Weight was measured shoeless and corrected for residual  
clothing weight.

(1) = Study 1. (2) = Study 2. (3) = Study 3.

TABLE 2. Altitude Chamber Temperature/Humidity Data

	Temperature	Humidity
No-Ozone Experiments:		
Mean	72° F	11.8%
Range	68°-74° F	11.5-12.5%
Ozone Experiments:		
Mean	72° F	11.8%
Range	70°-73° F	11.0-13.5%

TABLE 3. Schedule of Experimentation, First Study

Subject A		Subject B	
<u>Elapsed Time (min)</u>	<u>Activity</u>	<u>Elapsed Time (min)</u>	<u>Activity</u>
0	Report in/questionnaire Pulmonary function test Breakfast Electrode placement Urine void	0	Report in/questionnaire Pulmonary function test Breakfast Electrode placement Urine void
80-90	Lock - ascent		
90-105	No activity	95-105	Lock - ascent
105-120	Exercise	105-120	No activity
120-135	No activity	120-135	Exercise
135-150	Hand steadiness test	135-150	No activity
150-165	Exercise	150-165	Hand steadiness test
165-180	No activity	165-180	Exercise
180-210	Photopic testing	180-195	No activity
210-225	Exercise	195-225	Photopic testing
225-240	No activity	225-240	Exercise
240-300	Dark adaptation/ Scotopic testing	240-300	Dark adaptation/ Scotopic testing
300-315	Wechsler Memory test	300-315	No activity
315-330	Exercise	315-330	Wechsler Memory test
330-340	Lock - descent	330-345	Exercise
340	Pulmonary function test Urine specimen Blood sample Questionnaire Remove electrodes	345-355	Lock - descent
390	Release	355	Pulmonary function test Urine specimen Blood sample Questionnaire Remove electrodes
		405	Release

TABLE 4. Schedule of Experimentation, Second Study

Subject A		Subject B	
<u>Elapsed Time (min)</u>	<u>Activity</u>	<u>Elapsed Time (min)</u>	<u>Activity</u>
0	Report in/questionnaire Pulmonary function test Breakfast Electrode placement Urine void	0	Report in/questionnaire Pulmonary function test Breakfast Electrode placement Urine void
80-90	Lock - ascent		
90-105	No activity	95-105	Lock - ascent
105-120	Exercise	105-120	No activity
120-150	Photopic testing	120-135	Exercise
150-165	Exercise	135-165	Photopic testing
165-180	No activity	165-180	Exercise
180-225	Dark adaptation/ testing	180-225	Dark adaptation/ testing
225-240	Wechsler Memory test	225-240	No activity
240-255	Hand steadiness test	240-255	Wechsler Memory test
255-270	Exercise	255-270	Hand steadiness test
270-280	Lock - descent	270-285	Exercise
280	Pulmonary function test Urine specimen Blood sample Questionnaire Remove electrodes	285-295	Lock - descent
330	Release	295	Pulmonary function test Urine specimen Blood sample Questionnaire Remove electrodes
		345	Release

TABLE 5. Schedule of Experimentation, Third Study

Subject A		Subject B	
<u>Elapsed Time (min)</u>	<u>Activity</u>	<u>Elapsed Time (min)</u>	<u>Activity</u>
0	Report in/questionnaire Pulmonary function test Breakfast Electrode placement Urine void	0	Report in/questionnaire Pulmonary function test Breakfast Electrode placement Urine void
80-90	Lock - ascent		
90-105	No activity	95-105	Lock - ascent
105-120	Wechsler Memory test	105-120	No activity
120-150	Photopic testing	120-135	Wechsler Memory test
150-165	No activity	135-165	Photopic testing
165-210	Dark adaptation/ testing	165-210	Dark adaptation/ testing
210-220	Lock - descent	210-225	No activity
220	Pulmonary function test Urine specimen Blood sample Questionnaire Remove electrodes	225-235	Lock - descent
		235	Pulmonary function test Urine specimen Blood sample Questionnaire Remove electrodes
270	Release		
		285	Release

TABLE 6. Mean Experimental Ozone Levels\* (ppmv)

	Study 1	Study 2	Study 3
Mean	0.1953	0.2923	0.2909
Range	.1912-.1972	.2873-.2958	.2853-.2954
S.D.	0.0018	0.0027	0.0026
N	13	15	18

\* The values shown are means of the mean levels for each experiment. The ranges are the ranges of the means for each experiment. Ozone levels in the individual experiments were kept at the 0.20 or 0.30 ppmv levels most of the time; mean levels are lower because ozone generators were turned off during scotopic vision tests.



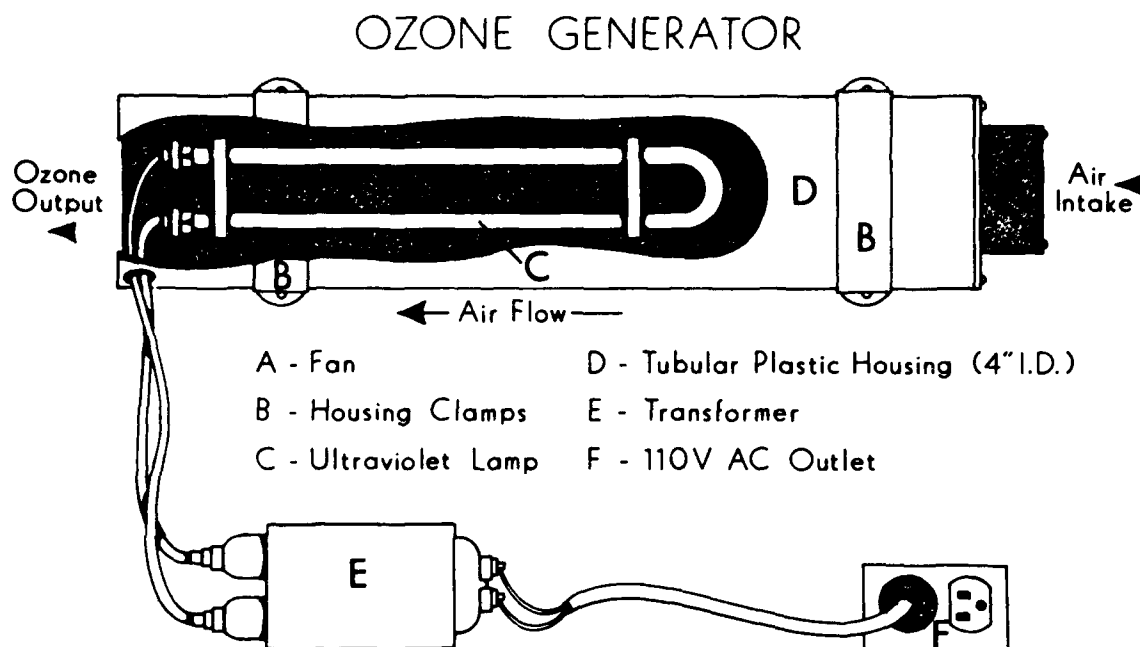


FIGURE 1. Diagrammatic representation of the ozone generator.

Presentation of ozone and no-ozone experimental conditions was balanced; i.e., half the subjects were exposed to ozone for their first experience and half were exposed to the no-ozone condition first. As it developed, this balancing of presentations was important because several physiological effects were related to the order of experiments and not to ozone.

The first two studies were designed to determine the threshold for subjective symptoms produced by, and physiological effects of, ozone on flight attendant surrogates. An attempt was made to provide a realistic in-flight environment as far as cabin pressure, humidity, temperature, and physical activity were concerned. When positive effects of 0.30 ppmv ozone were found on exercising subjects, the decision was made to determine the effects of that level of ozone on sedentary subjects. The third experiment, therefore, is relevant to ozone's effects on passengers and flight deck crews.

Specific details of experimental procedures, results, discussions, and summaries of the three studies are presented in the following sections.

Subjects were tested in pairs and were not informed about the experimental condition to which they were being exposed. No attempt was made to mask the odor of ozone or to conceal the generators from view because such procedures cause the breakdown of ozone and render it difficult if not impossible to maintain a desired concentration. Therefore, subjects were probably aware of the presence of ozone, though only one or two remarked about it.

In-chamber researchers wore plastic hoods perfused with air from a compressed air tank so that they would not be repeatedly exposed to ozone. Hoods were worn during both experimental conditions.

Atmospheric scientists commonly refer to a gas in terms of its "mixing ratio"; i.e., the volumetric proportion of a single gas to the total gas mixture. The mixing ratio may be expressed as percent, parts per thousand, parts per hundred thousand, parts per million, or parts per billion. However, when the biological effect of ozone is of interest it is not the proportion of ozone to other gases but its absolute amount that is important. As ambient pressure (altitude) changes, so does the molar concentration of ozone; the mixing ratio, however, stays constant, leading to a misrepresentation of the biologically effective dose. If the effects of more than one gas were being considered, it would be necessary to express the quantities of those gases in terms of mols/volume so that the effects could be related to the number of molecules involved. When only one gas is considered, as is the case in these experiments, it is equally acceptable and more convenient to express its quantity as mass/volume ( $\mu\text{g}/\text{m}^3$ ) or as pressure-corrected volume/volume. The International System of

Units (Système International d'Unités - SI) recommends that mass concentration expressions be standardized as  $\text{kg/m}^3$ ,  $\text{kg/L}$ ,  $\text{g/L}$ ,  $\text{mg/L}$ ,  $\mu\text{g/L}$ , or  $\text{ng/L}$  (1). Because ozone is biologically effective in such minute quantities compared to other constituent gases in air, the term  $\mu\text{g/m}^3$  yields a whole number and circumvents the use of fractional or decimal amounts. The aim of this report is to communicate the findings of this research to people of diverse backgrounds; therefore, the term "ppmv" is used preferentially throughout the report to express ozone concentration. The decision to use this expression primarily rather than  $\text{mols/L}$  or  $\mu\text{g/m}^3$  is based on the fact that "ppmv" is deeply ingrained in the language related to air pollution. The approximate relationship between altitude, ppmv, and  $\mu\text{g/m}^3$  of ozone is shown in Figure 2.

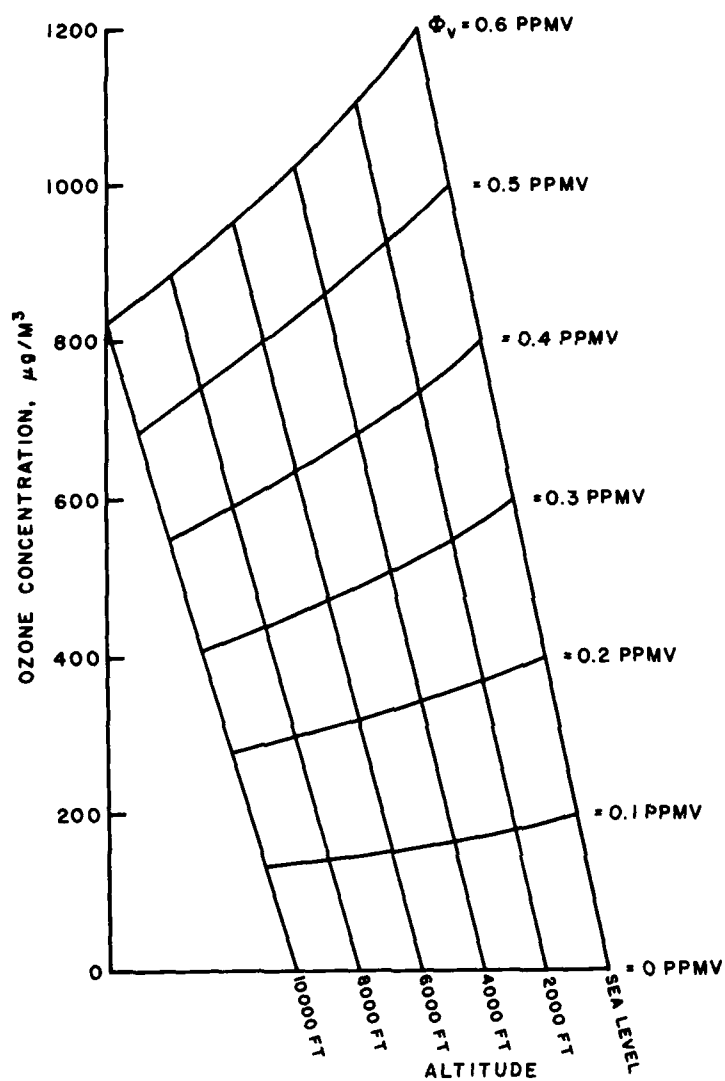


FIGURE 2. Graphic relationship between ppmv,  $\mu\text{g}/\text{m}^3$  and altitude.

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### SECTION III

#### EFFECTS OF OZONE ON HAND STEADINESS, HEART RATE, AND SHORT-TERM MEMORY

E. A. Higgins

##### Introduction.

In developing the protocols for the three ozone studies reported here, the following parameters were among those selected for measurement: hand steadiness, heart rate, and short-term memory. Of these three parameters, short-term memory was expected to be most susceptible to ozone effects because ozone has been reported to cause fatigue and to impair one's ability to concentrate (1). This lack of concentration could be a direct effect of ozone or it could be an indirect effect, perhaps psychogenic in origin, related to some of the symptoms credited to ozone exposure (1,2,3,4,5,6). It could be due to some degree of apprehension. Subjects were not told during which experiment they were exposed to ozone. However, particularly during Studies 2 and 3, the odor of ozone was quite noticeable inside the altitude chamber and subjects were able to distinguish between the experimental conditions. Some degree of apprehension during the ozone experiments might be anticipated because the possible harmful effects of ozone exposure were discussed with the subjects during their initial interviews in order to comply with "informed consent" procedures. Physical discomfort could be distracting and might also affect such parameters as hand steadiness. There is one report (7) of a persistent effect on coordination from a 2-hour, high level (1.5 to 2.0 ppm) ozone exposure.

Although no reports have been found in the literature which indicate a direct effect of ozone on heart rate, this appeared to be a logical measurement to include as an indicator of effects on the sympathetic nervous system. Physical discomfort experienced as a result of the ozone level would probably be reflected in this measurement.

##### Methods.

###### Hand Steadiness.

Hand steadiness was measured with the Motor Steadiness Kit (Marietta Apparatus Company). The subjects were required to center a probe in each of five holes that decreased in size from 0.44 to 0.28 cm in diameter; the probe was 0.20 cm in diameter. Subjects attempted to keep the probe centered in each hole for 30 s with a 30-s rest between attempts. The subjects were required to use one hand without support except for the forearm on the table. Subjects went through the sequence twice, each time starting with the largest

hole and ending with the smallest hole. The measure of steadiness was the number of contacts of the probe with the side of the holes accumulated on an electric counter (the greater the count, the poorer the steadiness).

#### Heart Rate.

Heart rate (HR) recordings were made on electromagnetic tape with chest electrodes in the CM<sub>5</sub> position connected to an Avionics Model 400 Electrocardiometer. Recordings were made by this method during the entire experimental period in Study 3 and during the experimental period except during treadmill exercise periods in Studies 1 and 2. The three studies are described in the General Methods section.

#### Wechsler Memory Scale.

This scale consists of seven subtests, the first three of which were not expected to contribute useful data to these studies. (Tests 1 and 2 are intended for use with subjects having special defects, such as aphasia. Test 3 consists of counting backward from 20 to 1, repeating the alphabet, and counting by 3s and 4s.)

Test 4, Logical Memory, consists of two memory passages similar to the memory selections on the 10th year of the Stanford-Binet and are similarly scored. The test is intended to measure immediate recall of logical material.

Test 5 is the familiar Memory Span for digits, forward and backward. The series used are those employed in the Wechsler-Bellevue Intelligence Scale except that the maximum number of digits used in the series is limited to eight forward and seven backward.

Test 6, Visual Reproduction, required the subject to draw simple geometric figures from memory after viewing a test figure for 10 s.

Test 7, Associate Learning, consists of 10 paired associates, some easy and some difficult; subjects are given three trials and the number of correctly recalled associates is the measure of performance.

The Wechsler Memory Scale comes in two equivalent forms. Form I was administered to the first of the two subjects to enter the chamber and Form II to the second subject during the first experiment. Each subject received the alternate form during the second experiment. The Wechsler Memory Scale yields a measure called the Memory Quotient (MQ) and is computed as follows: (i) Sum the subject's partial subtest scores. (ii) To this total, which is the subject's raw score, add a constant assigned for the age group in which the subject falls. This

new sum is the subject's weighted or corrected memory score. (iii) Obtain the equivalent quotient for this score from a table provided in the test manual. The value found is the subject's MQ corrected for age.

## Results.

### Hand Steadiness.

In the first two studies there were no statistically significant findings for hand steadiness for any comparisons made (ozone vs. no-ozone, male vs. female, or first vs. second experiment). It was decided, therefore, not to include this measurement in the third study.

### Heart Rate.

Data are presented in Figures 1 and 2. There were no statistically significant differences between ozone and no-ozone conditions for either sex or for the combined group in the three studies. However, during Study 2, females whose first exposure was to ozone demonstrated a significantly higher HR ( $p \leq 0.05$ ) during the ozone exposure than during the no-ozone exposure (Table 1).

TABLE 1. Mean Heart Rate (beats per minute), Second Study

<u>Exposure</u>	<u>Ozone First</u>	<u>No-Ozone First</u>
Males		
Ozone	70.0	74.6
No-ozone	71.0	74.1
Females		
Ozone	80.4*	81.0
No-ozone	76.3*	81.6

\* Statistically significant difference ( $p \leq 0.05$ ) ozone vs. no-ozone for those females who received the ozone exposure first.

Females exhibited a higher HR than males in all three studies; however, this difference was statistically significant ( $p \leq 0.05$ ) for the third study only. Only in Study 1 was there an order effect with the first experiment yielding a significantly higher HR ( $p \leq 0.05$ ) than the second experiment.



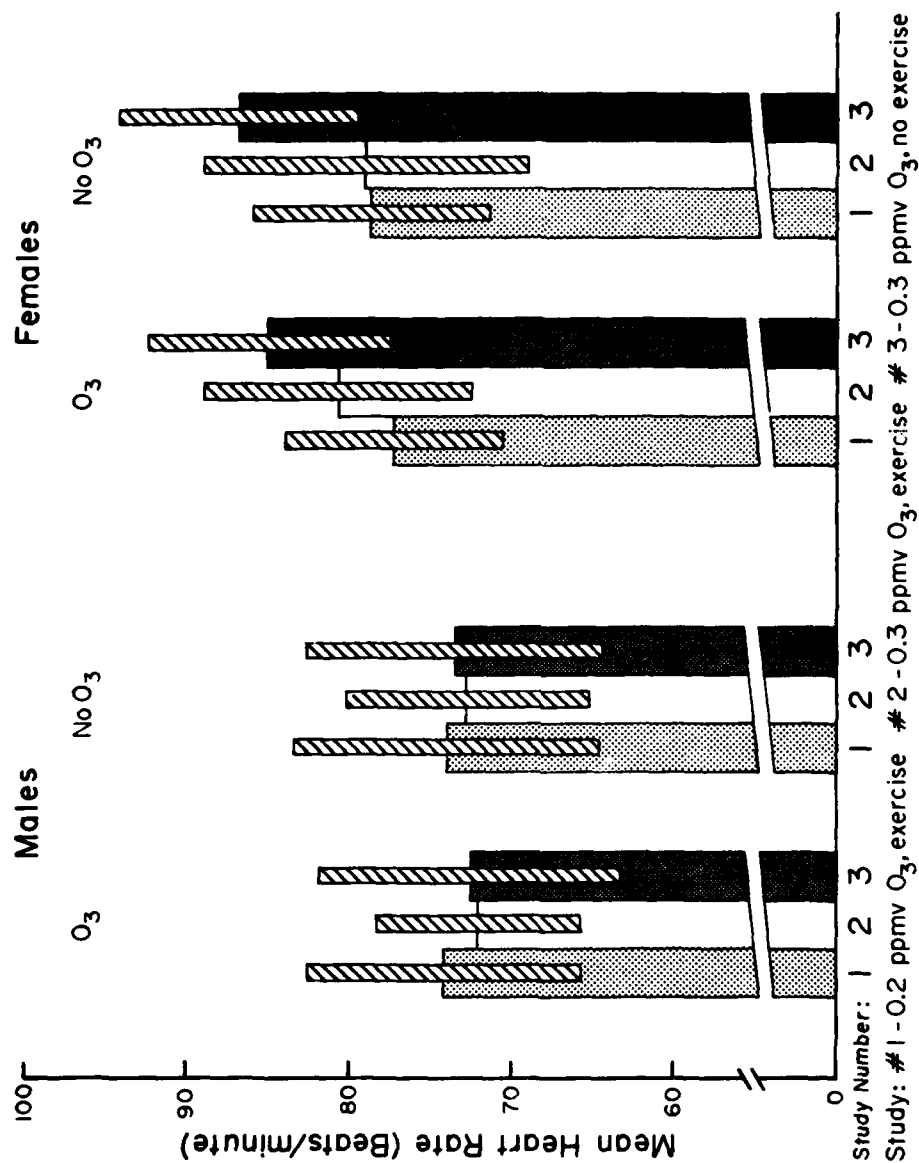


FIGURE 1. Bargraph of mean heart rate for the three ozone studies expressed as a function of ozone exposure vs. no-ozone exposure for males and females.

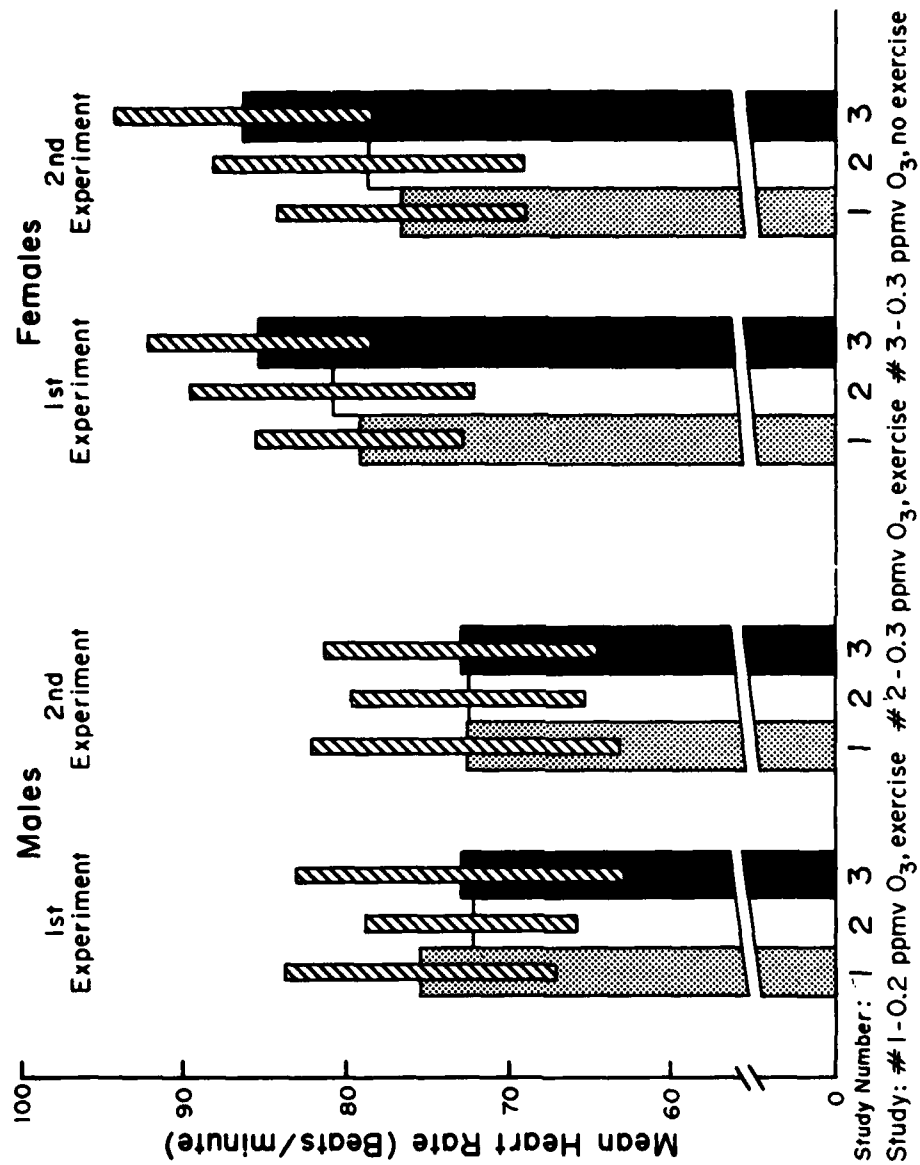


FIGURE 2. Bargraph of mean heart rate for the three ozone studies expressed as a function of order (first experiment vs. second experiment) for males and females.

### Wechsler Memory Scale.

There was a general trend for the MQ scores to be higher (better memory) during the no-ozone experiments than during the ozone experiments (Figure 3). Only the males in the second study did not follow this trend. In Study 2 there was a statistically significant difference in ozone exposure vs. no-ozone exposure for those females who received the ozone exposure first. No-ozone scores were higher (Table 2).

TABLE 2. Mean Wechsler MQ Scores, Second Study

<u>Exposure</u>	<u>Ozone First</u>	<u>No-Ozone First</u>
Males		
Ozone	115.9	117.3
No-ozone	116.4	113.1
Females		
Ozone	116.1*	115.3
No-ozone	124.7*	111.3

\* Statistically significant difference ( $p \leq .01$ ) ozone vs. no-ozone for females who received the ozone exposure first.

There was a statistically significant order effect (Figure 4) in Studies 2 and 3 ( $p \leq .05$ ). Although not of statistical significance, the trend also appeared in Study 1. The second test scores were consistently higher than the first test scores. In Study 3 there was a difference between the sexes with males scoring significantly higher than females ( $p \leq .01$ ).

### Discussion.

The hand steadiness measurement, executed in the first two studies, was unaffected by any of the variables (environment, experience, or gender) and was not included in the final study.

Heart rate was not affected by the presence of ozone when considering the full population in any of the three studies. In Study 2, however, the females who received the ozone exposure first did exhibit significantly higher HRs during exposure to ozone than during the no-ozone exposure. This finding may indicate that females are more sensitive to ozone than males. Also, the exercise imposed in Study 2 that was not in Study 3 was sufficient to cause the 0.30 ppmv ozone level for 3 h to be at the threshold for an effect on HR for these females.

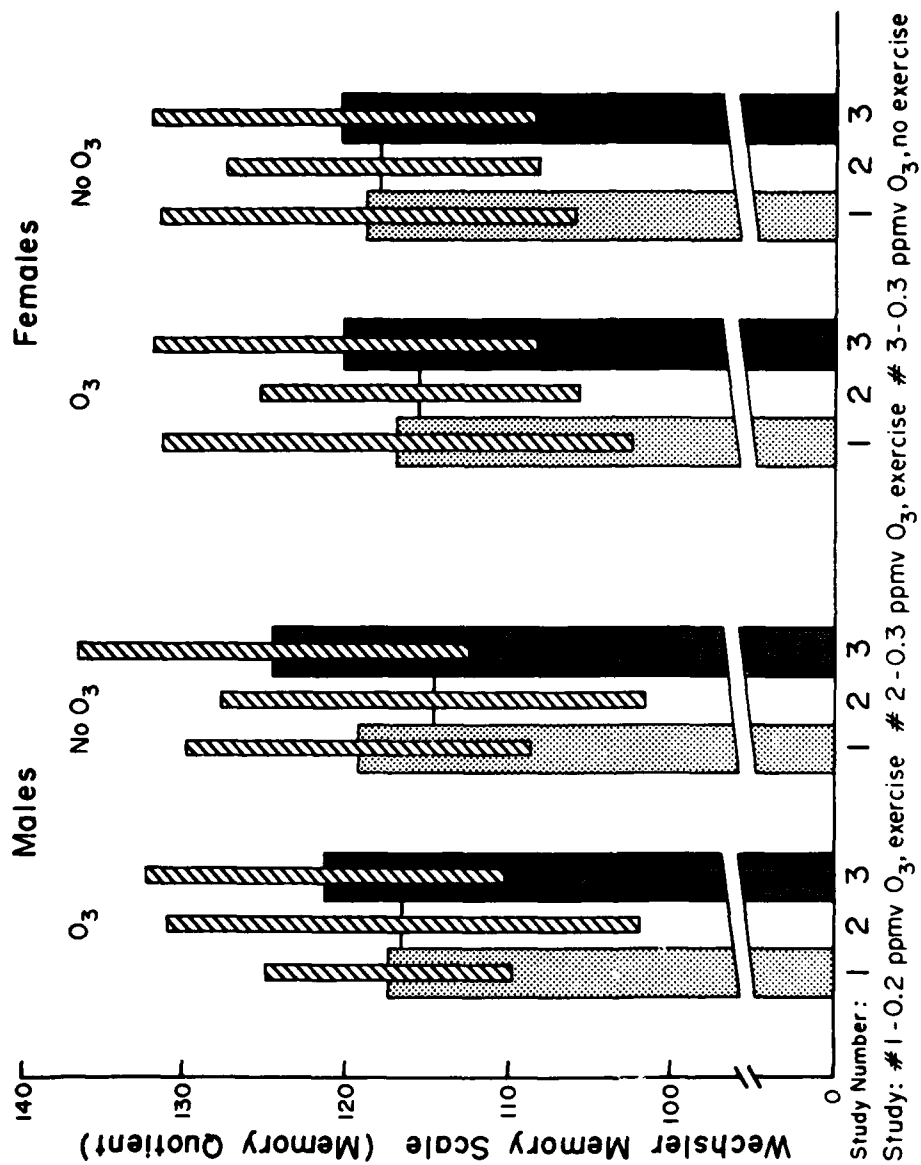


FIGURE 3. Bargraph of mean Wechsler Memory Score for the three ozone studies expressed as a function of ozone exposure vs. no-ozone exposure for males and females.

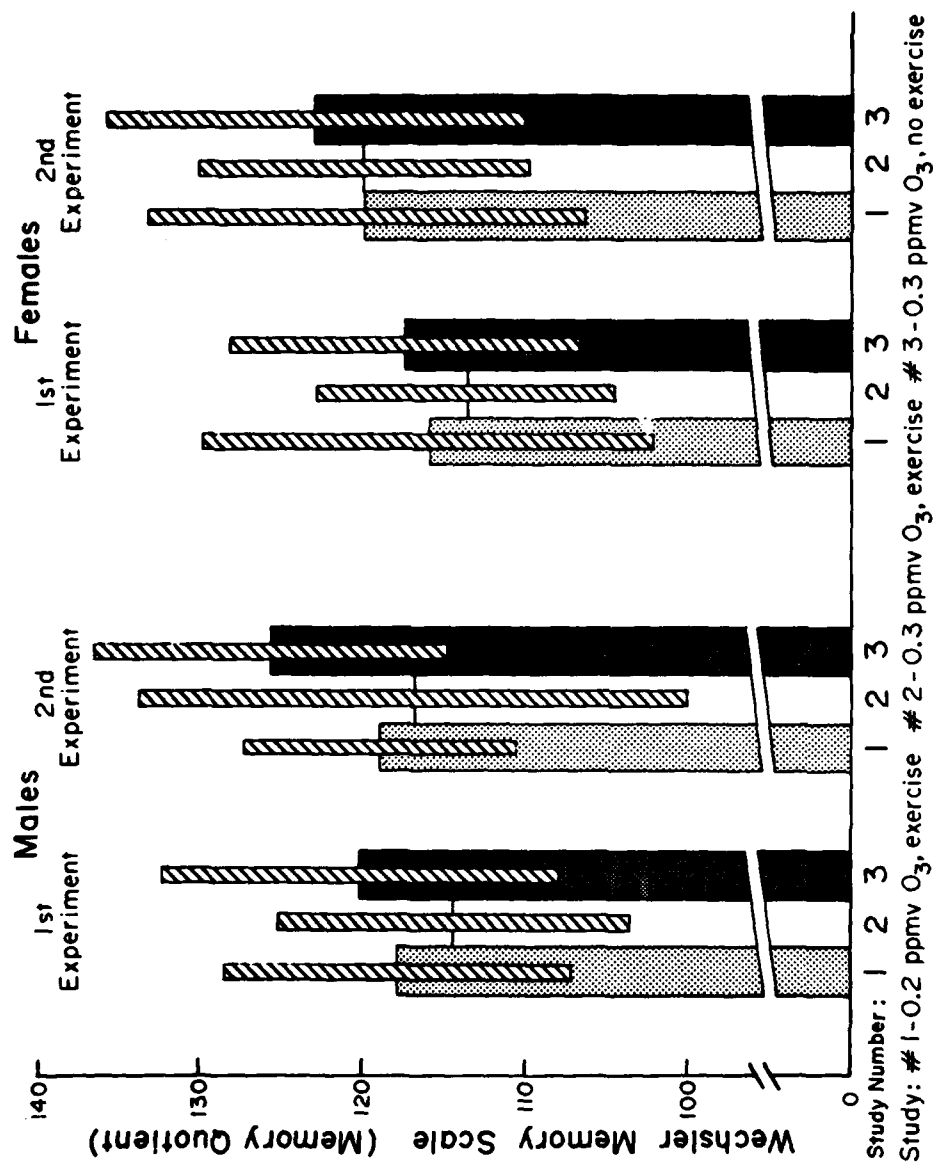


FIGURE 4. Bargraph of mean Wechsler Memory Score for the three ozone studies expressed as a function of order (first experiment vs. second experiment) for males and females.

The order effect on HR in Study 1 (HR higher in the first experiment than in the second) may indicate that these subjects were less apprehensive concerning the experimental protocol and more at ease during the second experiment.

Although females exhibited a higher mean HR than the males in all three studies, only in Study 3 was there a statistical difference. This difference was due primarily to the higher mean HR for the females in Study 3, 85.9 beats per minute (bpm) compared with 78.0 and 79.9 bpm for the females in Studies 1 and 2 respectively. The average HRs for the males were very consistent (74.1, 72.5, and 73.0 bpm for Studies 1, 2, and 3). It is possible that the female subjects in general were not as physically fit as the male participants, which might account for the higher HRs for females in all three studies. From conversations with subjects, the impression was that males were engaged in more physical activities (soccer, jogging, tennis, racquetball, physical conditioning, etc.) than the females. This, however, does not account for the significantly higher HR for the females in Study 3. No apparent explanation is readily evident at this time.

When considering the overall population in each of the three studies, ozone had no effect on short-term memory. Again, as with the HR, however, the females in Study 2 who were exposed to ozone first did demonstrate a statistically significant difference between the two environmental conditions. Their scores in the no-ozone experiments were much higher (8.6 points) than during the ozone experiments. If this were strictly an order effect (second exposure higher than the first), it would be expected that those females who received the no-ozone exposure first would have significantly higher scores during their no-ozone experiments. This was not the case. When examining the combined means for all three studies, there is a trend evident for MQ scores to be lower in the ozone exposures. For males, the combined mean MQ was 119.6 without ozone and 118.5 with ozone. For the females the mean MQ was 119.1 without ozone and 117.6 with ozone.

There was an order effect for the mean MQ scores with the scores in the second experiment in each study being higher than the scores in the first experiment. The difference was statistically significant for Studies 2 and 3. This finding is consistent with an earlier study (8). There was 1 week between taking the first Wechsler Memory Scale and the second. Apparently the experience gained in taking the first test provided some advantage in executing the second one.

The MQ scores for the three studies, by sex, are listed in Table 3.

TABLE 3. Mean Memory Quotient (MQ) Scores for the Three Studies

Group	Study Number			Mean
	1	2	3	
Males	118.5	115.7	122.5	119.0
Females	118.0	116.9	120.3	118.4
Combined	118.3	116.3	121.4	118.7

The time of administration of the Wechsler Memory Scale was not constant within the three studies. Studies 1 and 2 presented the Wechsler after completion of all vision testing. During Study 1, a 4-h chamber exposure, the Wechsler was administered 3 h, 30 min into the chamber exposure. In Study 2, a 3-h chamber exposure, the test was presented 2 h, 15 min into the chamber exposure. For the third study all vision tests were moved to the end of the experiment to provide as great an exposure period before vision testing as possible in case the negative findings for the vision tests during the first two studies were the result of a too-short exposure period prior to testing. Therefore, the Wechsler tests were administered 1 h, 15 min after exposure to the chamber environment. This may have some bearing on the lack of demonstrable findings for ozone vs. no-ozone in Study 3.

The significant difference in MQ scores between males and females in Study 3 was due to the very high scores obtained by the males in that group. It was not due to a low score by the females. In fact, the females in Study 3 scored higher than the females in the other two studies. The only conclusion is that, by chance, the group of 14 young men in Study 3 were exceptionally competent in the area of short-term memory.

#### Conclusions.

For the parameters reported in this section, ozone has no effect on heart rate, hand steadiness, or short-term memory, when presented for 4 h at 0.20 ppmv with four 10-min treadmill exercise sessions (Study 1), or when presented for 3 h at 0.30 ppmv without exercise (Study 3). Ozone at 0.30 ppmv for 3 h with three 10-min exercise sessions (Study 2) appears to be at the threshold level for effects on both heart rate and short-term memory for the females only.

Females, therefore, appear to be more susceptible to ozone effects than males and the addition of a physical workload lowers the ozone tolerance level for them.

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## SECTION IV

### CARDIOPULMONARY STUDIES AND SYMPTOM QUESTIONNAIRE

M. T. Lategola

#### Methods.

In Study 1, experimental orientation of the subjects consisted of a 2-h exposure to a chamber altitude of 6,000 ft MSL while breathing only ambient air. In Studies 2 and 3, the duration of this practice exposure was reduced to 45 min. During this altitude exposure, each subject experienced a practice run for each test to be employed during the actual subsequent experiments. For Studies 1 and 2, included in this altitude session was one 10-min treadmill test, during which a CM5 single-lead electrocardiogram (ECG) (1), HR, and pulmonary ventilation ( $\dot{V}_E$ ) were monitored. The treadmill load used was of moderate intensity and was equal to the last of each group of such tests to be used during each actual experiment. This treadmill test served the dual purpose of a practice session and a medical screen. The subject was disqualified if: (i) the HR exceeded 150 bpm; (ii) the single-lead ECG manifested ischemia and/or arrhythmia; or (iii) inability to easily tolerate the imposed load occurred. Since treadmill testing was omitted from Study 3, the treadmill practice was also omitted from its preceding altitude orientation session.

Each subject also underwent spirometric recording of at least three forced vital capacity (FVC) efforts immediately before and after the altitude orientation session. This procedure also served the dual purpose of practice and additional medical screening. The subject was disqualified if postaltitude spirometric function was abnormally displaced from that of the prealtitude assessment.

Each subject who remained qualified following the practice session was scheduled to return for two subsequent experiment sessions which were conducted 1 week apart.

The first procedure of the actual experiment session was a respiratory symptom questionnaire. The questionnaire, which is a slight modification of one used in a previous study (2), is shown in Figure 1. This questionnaire was administered to each subject by the same person throughout Studies 1, 2, and 3. The subject was first requested to perform one maximum volume inspiration and expiration, and was then queried concerning the presence and degree of discomfort in each of five symptom categories as listed in Figure 1. This procedure was completely repeated by the 10th min after exiting the altitude chamber lock as an assessment of immediate residual effects. The subject was then asked to recall the presence and degree of discomfort in each symptom category during

the last treadmill test at altitude (Studies 1 and 2) or during the whole altitude period (Study 3). The mathematical rating for each degree of discomfort is shown in Figure 1. The postaltitude symptomatic response was represented by the summation of the algebraic differences in each symptom category between the prealtitude and postaltitude assessments. The symptomatic response for the last treadmill test (Studies 1 and 2) or the whole altitude period (Study 3) was obtained by the same type of mathematical comparison with the prealtitude assessment. In this manner, each subject served as his/her own control in each experiment. Thus characterized, the symptomatic response of each subject in the no-ozone experiment was compared to his/her response in the ozone exposure experiment.

Following completion of the prealtitude questionnaire, each subject underwent spirometric assessment of mechanical pulmonary function. This consisted of recording a minimum of three maximal FVC efforts using a low-resistance 13.5 L Collins spirometer (Study 1) and a precalibrated electronic spirometer\* (Studies 2 and 3). During each FVC effort, the subject was seated in an upright position with a rubber nose clip in place. This FVC maneuver was conducted in accordance with a standard clinical spirometry procedure (3). The subject was allowed to rest for 1 min between maximum FVC efforts. The FVC, 1-s forced expired volume ( $FEV_1$ ),  $FEV_1/FVC \times 100$  ( $FEV_1\%$ ), forced midexpiratory flow ( $FEF_{25-75\%}$ ), and forced endexpiratory flows ( $FEF_{50-75\%}$  and  $FEF_{75-95\%}$ ) were measured from the data representing the best of three acceptable FVC efforts. The best effort was defined as that which yielded the largest sum of FVC +  $FEV_1$  (4). All volumes were expressed in liters, corrected to body temperature and pressure, saturated (BTPS). The  $FEF_{25-75\%}$ ,  $FEF_{50-75\%}$ , and  $FEF_{75-95\%}$  were included in this assessment because of their sensitivity in detection of changes in peripheral airway resistance (5,6). The  $FEF_{50-75\%}$  function was not assessed in Study 1 but was subsequently added to Studies 2 and 3. Spirometric assessment was repeated immediately after the exit of each subject from the altitude chamber lock. The postaltitude FVC response of each subject was expressed as percent of his/her prealtitude control value (postaltitude value/prealtitude value  $\times 100$ ). The postaltitude responses of the other five spirometry parameters were similarly calculated and expressed. In this manner, each subject served as his/her own control in each experiment. Thus characterized, the spirometric responses of each subject in the no-ozone experiment were compared to his/her responses in the ozone exposure experiment.

Preceding the ascent to altitude, three adhesive electrodes were attached to the chest of each subject for monitoring and recording a  $CM_5$  (manubrium to  $V_5$  plus ground) single-lead ECG (1) during each treadmill test. During each treadmill test at altitude, this ECG was fed simultaneously to: (1) an oscilloscope for continuous

\*Model M-10, automated spirometer, manufactured by SRL Medical, Inc., Dayton, Ohio.

visual monitoring for ischemia and/or arrhythmia; (ii) a cardio-tachometer for continuous meter indication of HR; (iii) a standard ECG recorder for scheduled periodic recording; and (iv) a pen-writing strip chart recorder (Grass Model 7) for subsequent HR measurements. The three chest electrodes were connected by shielded wires to a single phone jack that was plugged into the monitoring/recording system only during each treadmill testing procedure. The plug-in junction box was located inside the altitude chamber adjacent to the treadmill and all recording equipment was located on the outside of the chamber next to an observation window. At altitude at all other times except during the treadmill testing, the single-lead ECG was plugged into an Avionics ECG tape recorder for continuous recording of HR as described elsewhere in this report.

In Study 3, no treadmill tests were conducted during the 3-h altitude exposure. In Study 1, each subject underwent four treadmill tests during each 4-h altitude exposure, whereas in Study 2, each subject underwent three treadmill tests during each 3-h altitude exposure. Each treadmill test lasted 10 min and was preceded by a minimum of 30 min rest. The walking speed for all treadmill tests was set at 2.0 miles per hour (mph) for female subjects and at 3.0 mph for males. The treadmill incline was set at 5 percent for the last treadmill test, and at zero percent for all the others. The combinations of zero-percent incline with 2.0 mph for the females and 3.0 mph for the males were chosen as estimates for the production of a mean steady-state HR of about 100 bpm. The choice of these two combinations was based on the previous study of Astrand et al. in which the mean HR for 12 flight attendants (3 males and 9 females) during 4 h of in-flight duty was 108 bpm (7). The addition of the 5-percent incline to the last treadmill test (Studies 1 and 2) was chosen to generate a mean peak-load HR between 110 and 140 bpm in consonance with similar peak-load values also reported by Astrand et al. Besides relevance to actual flight attendant workloads the treadmill tests were included in these ozone studies because previous studies (8-11) have reported that adverse symptoms caused by ozone exposure are increased during exercise. This effect is presumably a function of increased ventilation caused by the exercise (11).

During each treadmill test, the HR and  $\dot{V}_E$  were recorded continuously on a multichannel recorder. The HR was obtained from the CM<sub>5</sub> single-lead ECG as described previously. Each subject wore a rubber nose clip and breathed through a respiratory check valve during each treadmill test. The expiratory port of the respiratory valve was connected via plastic hose to a mass flowmeter for continuous measurement of each expired breath. The analog signal from each expirate, as well as its

ramp integral, was recorded simultaneously on the multichannel recorder. In this manner, the  $\dot{V}_E$ , respiratory frequency (f) in respirations per min (rpm), and average tidal volume ( $V_T$ ) were obtained. For each treadmill test, mean values for each of these three parameters, as well as HR, were based on a steady-state sampling period consisting of the last 3 min of each test. The mass flowmeter and integrator were volume-flow calibrated at altitude prior to each experiment. All volumes were expressed in BTPS conditions existing in the altitude chamber. In order to normalize the volume data for differences in body size, both the  $\dot{V}_E$  and  $V_T$  were expressed as volume per kilogram of body weight. Thus tabulated, the data (HR,  $\dot{V}_E/\text{kg}$ ,  $V_T/\text{kg}$ , and f) for each treadmill test in the no-ozone experiment were compared to their counterparts in the ozone exposure experiment.

Because each subject used a valve mouthpiece during treadmill tests, oral communication by the subjects regarding discomfort or distress was precluded. Therefore, four simple hand signals were taught to each subject for his/her conveyance of "yes," "no," "everything is OK," or "stop the test" messages. Besides direct observation of each subject during each treadmill test by intra-chamber personnel, the subject was routinely asked at the 3d, 5th, and 7th min of each test if everything was OK. Each subject was given the clear unconditional option of stopping the experiment at any time of his/her choosing. Other single indications for immediate termination of the treadmill test (and the remainder of the experiment) were inappropriate dyspnea, audible wheezing, substernal chest pain, ataxic gait, general pallor, an HR exceeding 150 bpm, or ECG indications of ischemia and/or arrhythmia. These cessation criteria are somewhat standard in ozone experiments of this general type (2). A staff physician and emergency resuscitation equipment were immediately available in case of need.

### Results.

To compensate for any potential effects due to experimental order, half the number of males and females in each of the three studies underwent the ozone exposure in the first experiment, and the remaining half underwent the ozone exposure in the second experiment. The data were pooled within each sex group and compared on the basis of no-ozone versus ozone exposure.

#### Symptom Questionnaire.

The mean scores for subjective symptoms in the no-ozone and ozone exposure experiments of Studies 1, 2, and 3 are summarized in Table 1.

In Study 1, none of the differences between the ozone and no-ozone mean scores were statistically significant. The highest mean score (6.7) lay between "trace" and "slight" in terms of degree of discomfort (see Figure 1).

In Study 2, the differences between the no-ozone and ozone mean scores within each sex group were statistically significant ( $p \leq 0.05$ ) for the immediate postaltitude period as well as during the last treadmill test at altitude. The highest mean score (16.8) occurred in the male group during the last treadmill test. This mean score lay between "slight" and "moderate" in terms of discomfort degree (see Figure 1). Although less than 16.8, the remaining three mean scores (males and females) also lay between "slight" and "moderate" discomfort.

In Study 3, none of the differences between the ozone and no-ozone mean scores were statistically significant. The highest mean score (14.6) occurred in the female group during altitude exposure. This mean score lay between "slight" and "moderate" discomfort.

In Studies 1, 2, and 3 no experiment had to be terminated because of intolerable symptomatic stress on the part of any subject.

#### Spirometry.

The mean values representing displacement of all the spirometry parameters in the ozone and no-ozone experiments of Studies 1, 2, and 3 are summarized in Table 2 (males) and Table 3 (females).

In Study 1, none of the differences between the ozone and no-ozone mean values for either sex group were statistically significant.

In Study 2, the differences between the no-ozone and ozone responses for all of the spirometry parameters within each sex group were statistically significant ( $p \leq 0.05$ ). As seen in both tables (2 and 3), postaltitude spirometric function increased over the prealtitude control values in the no-ozone experiments and decreased in the ozone exposure experiments. In each sex group, the three volume parameters (FVC, FEV<sub>1</sub>, and FEV<sub>1</sub>%) manifested smaller changes than the three volume-flow parameters.

In Study 3, with the single exception of the FEF<sub>50-75%</sub> parameter in the males, none of the differences between the no-ozone and ozone responses for all of the remaining spirometry parameters of each sex group were statistically significant. As in Studies 1 and 2, the increases in postaltitude spirometry values of the no-ozone experiments always exceeded those of the ozone exposure experiments.

### Treadmill Tests.

Heart rate,  $\dot{V}_E/\text{kg}$ ,  $V_T/\text{kg}$ , and  $f$  data for each treadmill test in no-ozone and ozone exposure experiments of Studies 1, 2, and 3 are summarized in Table 4 (males-Study 1), Table 5 (females-Study 1), Table 6 (males-Study 2), and Table 7 (females-Study 2). As mentioned previously, no treadmill tests were conducted in Study 3. For all four treadmill parameters in each treadmill test and in each sex group, none of the differences between ozone and no-ozone mean values were statistically significant (all  $p$  values  $> 0.05$ ).

ECG indications of ischemia and/or arrhythmia were completely absent during all of the treadmill tests performed in Studies 1 and 2.

### Discussion and Summary.

#### Symptom Questionnaire.

In Study 1, in which no statistically significant ozone symptoms occurred, the distribution and degrees of symptoms were about as varied in the no-ozone experiments as they were in the ozone exposure experiments. The most frequent single discomfort reported was throat dryness during the last treadmill test, and this occurred in six subjects during the no-ozone experiments and in an equal number during the ozone exposure experiments. Since the intrachamber relative humidity was held fairly constant in both the ozone and no-ozone experiments, the throat dryness occurring during the last treadmill test was probably not ascribable to ozone but to the increased oral ventilation of very dry air.

In Study 2, in which statistically significant symptomatic displacements occurred, postaltitude symptom mean scores in the ozone experiments were equally substantial for both sex groups. The commensurate postaltitude scores in the no-ozone experiments revealed only insignificantly small displacements. Since the postaltitude symptom assessment was completed by the 10th min after exiting the altitude chamber lock, and since an additional 10 min were spent in descent, a total of 20 min intervened between this symptom assessment and the actual cessation of ozone exposure. Since recovery from completely reversible degrees of ozone symptoms is known to occur somewhat rapidly following cessation of ozone exposure (12), the degree of symptomatic discomfort during the ozone exposure at altitude probably exceeded that which existed during the postaltitude assessment. This probability is generally supported by the higher symptom mean scores that occurred during the last treadmill test at altitude (Table 1).

During the last treadmill test, the symptom mean scores for the no-ozone and ozone experiments (Table 1) were greater in the males

than in the females. This was most probably due to the greater  $\dot{V}_E/\text{kg}$  in the males as compared to the females (see Tables 6 and 7). As reflected in the HR data of the last treadmill test (Tables 6 and 7), the increased  $\dot{V}_E/\text{kg}$  in the males was a result of the relatively greater workload in the males (mean HR about 115 bpm) as compared to the females (mean HR about 110 bpm).

In Table 1, because the symptom mean score during the last treadmill test in the no-ozone experiments is greater in the males than in the females, this indicates that low humidity, per se, may be respiratorily irritating in roughly direct proportion to increases in oral instead of oronasal ventilation. It is well known that the nasal passages are far more efficient than the mouth in warming and wetting inspired air. The nasal passages are also more efficient than the mouth as a scrubber of ozone from inspired air (13,14).

Although the symptom mean score during the last treadmill test in the ozone exposure experiments (Table 1) was greater in the males than in the females, the difference between the no-ozone and ozone mean scores was slightly greater in the females (10.7) as compared to the males (10.4). These differences were presumably due to the effects of the ozone, per se, since altitude and low humidity were held constant in both the ozone and no-ozone experiments. The fact that this degree of symptomatic displacement (10.7 versus 10.4) was 2.9 percent greater in the females, even though the  $\dot{V}_E/\text{kg}$  of the females during the last treadmill test was about only 65 percent of the commensurate  $\dot{V}_E/\text{kg}$  of the males, suggests the strong probability that this group of females was more symptomatically sensitive to ozone than the males. Since ozone symptoms are known to increase when ventilation is increased by physical exertion (8-11), increasing the  $\dot{V}_E/\text{kg}$  of the females to an equivalency with the males would most probably increase the female ozone symptoms by some amount. If present, a greater ozone sensitivity in the females assumes greater importance, because the majority of flight attendants are female.

If the workload HR data of Astrand et al. (7) are assumed as a valid average representation for flight attendants during 4 h of flight duty, then the degrees of symptomatic and spirometric displacements observed in this study were elicited by a far lesser workload. The average HR for the 4-h work shift of Astrand et al. (7) was 108 bpm. At most, in our Study 2, the HR approximated this value for only 30 min of the 3-h exposure. Since it has already been established that the additional ventilation of physical activity increases the symptomatic effects of ozone exposure (8-11), a threefold increase in the activity duration of our subjects would most probably have increased the degree of ozone symptoms. This hypothetical threefold increase in activity duration was chosen to project a total time of 2 h of ozone exposure at an HR of about 108 bpm,

because 2 h has been projected as the maximum expected exposure time to 0.30 ppmv ozone in a maximum flight duration of 3 h. Therefore, according to a previously defined threshold level for ozone symptoms (15), that threshold was clearly exceeded by a 3-h exposure to 0.30 ppmv ozone at 6,000 ft MSL altitude. If a 2-h altitude exposure to ozone at an average HR of 108 bpm is more reflective of the safe maximum allowable environmental condition, then the ozone concentration threshold for symptoms is probably  $< 0.30$  ppmv, but  $> 0.20$  ppmv.

Qualitatively, symptoms were mainly felt in the throat, substernum, and eyes. Throat irritation was the most prevalent symptom and seemed to be characterized by involuntary coughing, especially during and following the maximum FVC efforts of spirometry testing. The symptom of second prevalence was substernal tightness, pain or ache, and was closely followed in prevalence by dry or burning eyes. This latter symptom was more prevalent during the last treadmill test than it was during the postaltitude assessment. Of all subjects tested, only two males experienced coughing during the last treadmill test. Only two males and three females manifested no increase in their symptom scores following ozone exposure as compared to their commensurate no-ozone scores.

As a procedural addendum, all subjects experiencing postaltitude symptoms were queried via telephone on successive days until the disappearance of all symptoms had occurred. One male complained of substernal discomfort (especially with deep breathing) for 3 subsequent days. One female reported a continued throat irritation and cough for 6 h after her departure. Two males and two females reported a considerable degree of lassitude for 2 subsequent days. All other subjects manifesting any symptoms reported that all symptoms had disappeared in 4 h or less. Symptoms due to this degree and duration of a single ozone exposure may probably be considered as being reversible.

In Study 3, although a "slight" to "moderate" degree of symptoms occurred during the ozone exposure at altitude, none of the differences between the ozone and no-ozone mean scores were statistically significant.

As shown in Table 1, the algebraic differences between the ozone and no-ozone symptom mean scores for the females in both the altitude and postaltitude assessments were greater than the commensurate differences for the males. Since this greater difference in symptom mean scores for the females is consistent with the same general finding as reflected in the symptom data of Study 2 (Table 1), the probability of a disproportionately greater ozone sensitivity in the females appears to be strengthened.

Eye discomfort was the most prevalent symptom in Study 3, as contrasted with the more prevalent throat and substernal symptoms of



Study 2. This apparent discrepancy between two identical-duration exposures to 0.30 ppmv ozone at altitude is probably explained by two differences in experimental protocol. First, the presence of three treadmill tests in Study 2 and no treadmill tests in Study 3 may have relatively accentuated throat and substernal symptoms in the former study because of the substantially increased oral ventilation occurring during the treadmill tests. Second, the occurrence of eye testing toward the end of the 3-h altitude/ozone exposure in Study 3 and toward the beginning of the altitude/ozone exposure in Study 2 may have relatively accentuated eye irritation in the former study as a function of the greater duration of ozone exposure preceding the eye testing. In descending order, headache and nasal burning were the next two most prevalent ozone symptoms in Study 3. As compared to Study 2, the greater prevalence of nasal burning in Study 3 may have resulted from relatively more nasal breathing in the latter study. Under the relatively sedentary conditions of Study 3, nasal breathing would have a greater probability of predominance than either oronasal or oral breathing. If nasal breathing did predominate in Study 3, it could explain some of the diminution of thoracic symptoms and spirometry displacements of Study 3 as compared to Study 2. The presence of nasal scrubbing of ozone (13,14) may have actually reduced the intrapulmonic exposure to ozone in Study 3, whereas the nonoptional switch from nasal to increased oral breathing during the treadmill tests of Study 2 may have relatively accentuated the intrapulmonic exposure to ozone. If generally true, this deduction suggests that, in the presence of an unavoidable exposure to ozone, some degree of intrapulmonic protection might be afforded by breathing only through the nose. This temporary expedience may have increased significance for individuals who may have increased intrapulmonic sensitivity to ozone due to such conditions as asthmatic allergies.

Following ozone/altitude exposure, 10 males and 7 females in Study 3 were completely free of symptoms during the postaltitude symptom assessment. In most of the remaining subjects, all symptoms had disappeared within 1-4 h of their postexperimental departure. Exceptions to this were: one male complaint of throat irritation for the remainder of the day; one female and one male with minor throat symptoms for 2 ensuing days; one female with a headache for the remainder of the day; and one female who developed slight postexperimental symptoms of headache, appetite loss, and fatigue for the remainder of the day. All of these symptoms may probably be considered as being reversible.

#### Spirometry.

In Study 1, The FEF<sub>75-95%</sub> data (Tables 2 and 3) indicate that postaltitude mean values increased over their corresponding prealtitude

values. This could have resulted from a mechanical decrease in peripheral airway resistance due to the increased  $V_T$  which usually accompanies exercise. Assuming constance of respiratory dead space, any increase in  $V_T$  would tend to increase parenchymal exposure to ozone. If one assumes that such exposure to ozone did occur, then the statistically insignificant differences in spirometric function between the ozone and no-ozone experiments indicate that this 4-h exposure to 0.20 ppmv ozone at altitude did not breach the threshold (15) for adverse spirometric effects in this nonsmoking population sample.

In Study 2, all of the postaltitude spirometry parameters in the no-ozone experiments increased over their commensurate pre-altitude values. This may have resulted from a "warming up" effect of the treadmill exercise on mechanical pulmonary function. All of the commensurate postaltitude mean values in the ozone exposure experiments manifested a decrease. Because many of the symptoms reflected discomfort in the upper tracheobronchial portion of the lung, the somewhat small but statistically significant changes in FVC,  $FEV_1$ , and  $FEV_1\%$  were not unexpected. However, because none of the subjects complained of any pain or discomfort in the more peripheral portions of the lung, the larger and statistically significant changes in the three FEF parameters were unexpected. This lack of symptoms at a time when substantial decreases in peripheral airway conductances are occurring may be of importance. One of the serious effects of acute severe ozone exposure is pulmonary edema (16). If peripheral pulmonary symptoms are either absent or insensitive right up to the point of edema, then the symptomatic warning of approach to this condition is also absent. In our study, since all of the treadmill parameters in the ozone experiments were not significantly displaced from those of the no-ozone experiments, the subjects were most probably in no danger of this condition. However, the possibility exists that the increased ventilation concomitant with a sustained average HR of about 108 bpm for 2 h at altitude and 0.30 ppmv ozone could affect pulmonary function in the increased direction of edema. The exact etiology of this type of edema is not known (16). In man, it has been reported that there may be a latent period of several hours before edema is detectable (16). Therefore, the removal of any person from experimental ozone exposure at the first definite symptoms of throat and/or substernal discomfort would appear to be prudent. In Study 2, every subject experienced a decrease in one or more of the six spirometric parameters as a result of the exposure to ozone. In terms of the spirometric parameters assessed, the threshold for an ozone effect on mechanical pulmonary function

(16) was clearly exceeded by the 0.30 ppmv ozone exposure of this study. In reasoning parallel to that employed in the symptom section of this discussion, the threshold for mechanical pulmonary effects of ozone most probably lies between 0.20 and 0.30 ppmv.

In Study 3, although differences between the ozone and no-ozone responses for all of the spirometry parameters of each sex group were qualitatively present, only that of the FEF<sub>50-75%</sub> parameter in the males reached statistical significance. It was reasonable to expect less separation between the ozone and no-ozone spirometric displacements in Study 3 as compared with Study 2, since no treadmill tests were conducted during the ozone/altitude exposures of Study 3. The fact that qualitative differences between the ozone and no-ozone responses did occur in Study 3 and that one function difference reached statistical significance probably means that a 3-h exposure to 0.30 ppmv ozone at altitude under somewhat sedentary conditions is right at the threshold (16) for spirometry effects of ozone.

#### Treadmill Tests.

In other studies, in which exercise was used in combination with ozone exposure at ground level (2), the workload imposed was that which approximately doubled resting ventilation. In all of the experiments of Studies 1 and 2, a trebling of the resting ventilation was exceeded in the last treadmill test, and a doubling exceeded in all of the preceding ones. The increased ventilation over resting levels was achieved by increases in both  $f$  and  $V_T$ . This increased ventilation along with oral breathing through a respiratory valve most probably guaranteed a reasonably good exposure of both the pulmonary parenchyma and airways to the inhaled ozone.

In Study 1, each subject underwent a total of 40 min of treadmill walking in each of two 4-h experiments. The average HR (Tables 4 and 5) during each treadmill test approached the targeted mean values for all 4 h of flight duty as reported by Astrand et al. (7). Since adverse effects of ozone exposure are known to be accentuated by exercise (8-11), the question of whether or not adverse effects would have emerged with a mean HR of about 108 bpm for the whole 4 h of the exposure to 0.20 ppmv ozone at altitude remains unanswered. The conservative amount of only 40 min (total) of treadmill walking per experiment was chosen primarily with subject safety in mind because of the uncertainty regarding the degree of adverse synergism, if any, of ozone exposure combined with altitude and relatively dry inspired air.

In Study 2, each subject underwent a total of 30 min of treadmill walking in each of two 3-h experiments. A trend of increased  $\dot{V}_E/kg$ , increased  $f$ , and decreased  $V_T/kg$ , as reported in other studies utilizing

ozone concentrations greater than 0.30 ppmv (8-12), was only segmentally present in our data as seen in Tables 6 and 7. The conjecture that this type of data trend may have increased toward significance in this study with 2 h of subjection to altitude and 0.30 ppmv ozone at an average HR of 108 bpm (7) must remain moot. However, it can be concluded that the symptom and spirometry parameters of Study 2 were more sensitive than the treadmill parameters as initial indicators of the adverse effects of the ozone exposure.

#### Summary.

Under essentially sedentary conditions of altitude exposure, the threshold for reversible adverse effects of ozone on objective spirometry functions appears to be right at a 3-h exposure to 0.30 ppmv. Populationwise, this would be relevant to passengers and sedentary aircrew. Under conditions of altitude and physical activity commensurate with average flight attendant workloads, the threshold for reversible adverse effects of ozone on subjective symptoms and objective spirometry functions appears to be exceeded by a 3-h exposure to 0.30 ppmv. Hypothetical interpolation of the data to a 2-h altitude exposure at an average HR of 108 bpm (7) would appear to place the ozone threshold between 0.20 ppmv and 0.30 ppmv for flight attendants. When the degree of subjective symptoms of the males and females were compared on the basis of relative activity levels (HR and  $\dot{V}_E/\text{kg}$ ), there emerged a suggestive strong probability that greater symptomatic sensitivity to ozone exists in the female.

SUBJECT #	A	B	INITIALS	DATE	OZONE	NO-OZONE
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SYMPTOM CHECKLIST	LAST TREADMILL TEST AT ALTITUDE (STUDIES 1 AND 2) OR	
	PREALTITUDE	ALTITUDE ONLY (STUDY 3)
		POSTALTITUDE

DISCOMFORT IN:

1. Throat
2. Under Breastbone
3. Chest (Other Than #2)
4. Headache
5. Other

RATINGS FOR DEGREE OF SYMPTOMATIC DISCOMFORT:

0 = None.      5 = Trace = Unsure of presence of discomfort.

10 = Slight = Symptom present but not annoying.

20 = Moderate = Symptom present and annoying.

30 = Severe = Symptom present and clearly painful.

FIGURE 1. Symptom questionnaire.

TABLE 1. Subjective Symptom Scores

		(Postaltitude)- (Prealtitude)		(Last (Treadmill Test)- (Prealtitude)		(Altitude)- (Prealtitude)	
		Ozone	No- Ozone	Ozone	No- Ozone	Ozone	No- Ozone
(1) M	$\bar{X}$	6.7	0.7	5.0	5.0		
	SE	3.0	1.5	1.9	2.2		
(1) P		0.084		1.000			
(1) F	$\bar{X}$	0.8	0.8	3.8	2.9		
	SE	1.0	2.0	2.0	1.8		
(1) P		1.000		0.760			
(2) M	$\bar{X}$	12.1	1.4	16.8	6.4		
	SE	2.4	0.8	2.4	2.3		
(2) P		< 0.001*		0.005*			
(2) F	$\bar{X}$	12.1	0	13.6	2.9		
	SE	3.2	0	2.9	1.6		
(2) P		0.001*		0.003*			
(3) M	$\bar{X}$	2.5	0.4			11.4	4.6
	SE	1.3	0.4			2.3	2.5
(3) P		0.116				0.056	
(3) F	$\bar{X}$	5.4	2.1			14.6	5.0
	SE	2.5	1.6			3.9	3.6
(3) P		0.290				0.081	

M = male. F = female.  $\bar{X}$  = mean. SE = standard error of the mean.  
P = statistical probability value. A statistically significant difference is defined as a value of  $p \leq 0.05$ . (1) = Study 1. (2) = Study 2.  
(3) = Study 3. \* = statistically significant difference.

TABLE 2. Spirometry Data (Males)

$$\frac{(\text{Postaltitude Value})}{(\text{Prealtitude Value})} \times 100$$

		FVC	FEV <sub>1</sub>	FEV <sub>1</sub> /FVC x 100	FEF <sub>25-75%</sub>	FEF <sub>50-75%</sub>	FEF <sub>75-95%</sub>
(1) Ozone	$\bar{X}$	99.4	99.7	100.5	98.5		105.3
	SE	0.5	0.7	1.4	1.3		3.2
(1) No-Ozone	$\bar{X}$	100.5	100.7	100.2	100.8		108.5
	SE	0.4	0.6	0.4	1.0		2.7
(1) P		0.384	0.322	0.204	0.845		0.444
(2) Ozone	$\bar{X}$	98.4	97.4	98.9	94.8	90.2	95.2
	SE	0.6	0.8	0.4	1.3	1.0	2.1
(2) No-Ozone	$\bar{X}$	101.2	101.7	100.6	102.8	103.4	107.6
	SE	0.6	0.4	0.4	0.6	1.1	2.2
(2) P		0.003*	< 0.001*	0.009*	< 0.001*	< 0.001*	< 0.001*
(3) Ozone	$\bar{X}$	99.6	100.2	100.6	102.7	101.2	103.1
	SE	0.3	0.8	0.6	1.0	0.9	2.9
(3) No-Ozone	$\bar{X}$	100.4	102.1	101.6	104.9	106.5	108.4
	SE	0.3	0.7	0.6	1.2	2.0	3.0
(3) P		0.055	0.092	0.261	0.165	0.021*	0.216

All symbols have been defined previously in Table 1 or the text. \* = statistically significant difference.

TABLE 3. Spirometry Data (Females)

$$\frac{(\text{Postaltitude Value})}{(\text{Prealtitude Value})} \times 100$$

		FVC	FEV <sub>1</sub>	FEV <sub>1</sub> /FVC x 100	FEF <sub>25-75%</sub>	FEF <sub>50-75%</sub>	FEF <sub>75-95%</sub>
(1) Ozone	$\bar{X}$	99.7	99.4	99.7	99.0		102.3
	SE	0.5	0.5	0.4	3.3		3.4
(1) No-Ozone	$\bar{X}$	100.3	102.5	100.1	102.7		111.4
	SE	0.6	2.0	0.5	1.2		5.7
(1) P		0.823	0.184	0.145	0.582		0.201
(2) Ozone	$\bar{X}$	98.0	97.5	99.4	96.6	91.5	92.0
	SE	0.7	0.9	0.4	1.9	3.7	1.9
(2) No-Ozone	$\bar{X}$	101.7	102.1	100.7	103.1	107.1	105.3
	SE	0.7	0.9	0.4	0.3	5.4	3.5
(2) P		0.001*	0.001*	0.030*	0.026*	0.003*	0.003*
(3) Ozone	$\bar{X}$	99.6	100.1	100.6	100.3	100.0	97.5
	SE	0.5	0.5	0.4	1.5	1.6	2.2
(3) No-Ozone	$\bar{X}$	101.0	100.9	100.0	101.8	101.8	101.9
	SE	0.5	0.4	0.4	1.3	1.4	2.7
(3) P		0.065	0.237	0.322	0.437	0.409	0.331

All symbols have been defined previously in Table 1 or the text. \* = statistically significant difference.



TABLE 4. Treadmill Data (Males - Study 1)

			HR (bpm)	$\dot{V}_E/\text{kg}$ (mL/min/kg)	f (rpm)	$V_T/\text{kg}$ (mL/kg)
TMT 1	Ozone	$\bar{X}$	102.5	276.2	16.5	18.8
		SE	3.5	10.4	1.5	1.7
	No-Ozone	$\bar{X}$	102.3	277.9	17.1	17.6
		SE	2.9	12.0	1.3	1.5
TMT 2	Ozone	$\bar{X}$	101.0	269.8	17.1	17.1
		SE	3.4	10.6	1.3	1.5
	No-Ozone	$\bar{X}$	100.6	270.8	17.6	16.5
		SE	2.7	10.8	1.2	1.4
TMT 3	Ozone	$\bar{X}$	99.1	282.4	18.0	16.9
		SE	3.4	10.9	1.4	1.4
	No-Ozone	$\bar{X}$	99.2	278.4	18.2	16.1
		SE	2.8	10.6	1.0	1.1
TMT 4	Ozone	$\bar{X}$	116.0	419.4	19.1	23.2
		SE	4.3	15.9	1.3	1.7
	No-Ozone	$\bar{X}$	116.5	423.5	20.0	22.0
		SE	4.0	17.5	1.1	1.5

TMT = Treadmill Test. All other symbols have been defined previously in Table 1 or the text.

TABLE 5. Treadmill Data (Females - Study 1)

			HR (bpm)	$\dot{V}_E/\text{kg}$ (mL/min/kg)	f (rpm)	$V_T/\text{kg}$ (mL/kg)
TMT 1	Ozone	$\bar{X}$	98.1	225.3	16.1	15.3
		SE	2.4	11.9	1.4	1.7
	No-Ozone	$\bar{X}$	100.2	244.6	16.4	15.6
		SE	2.5	10.8	1.1	1.2
TMT 2	Ozone	$\bar{X}$	95.7	219.6	15.8	15.5
		SE	2.5	10.1	1.3	1.9
	No-Ozone	$\bar{X}$	97.1	240.4	17.1	14.7
		SE	2.6	11.0	1.1	1.1
TMT 3	Ozone	$\bar{X}$	94.2	231.0	16.2	15.6
		SE	2.6	11.7	1.2	1.8
	No-Ozone	$\bar{X}$	95.2	245.4	16.7	15.5
		SE	2.9	11.3	1.1	1.2
TMT 4	Ozone	$\bar{X}$	106.7	296.4	18.2	17.5
		SE	2.1	12.7	1.3	1.6
	No-Ozone	$\bar{X}$	108.0	313.4	18.8	17.5
		SE	2.5	16.0	1.2	1.3

All symbols have been defined previously in Table 1, Table 4, or the text.

TABLE 6. Treadmill Data (Males - Study 2)

			HR (bpm)	$\dot{V}_E/\text{kg}$ (mL/min/kg)	f (rpm)	$V_T/\text{kg}$ (mL/kg)
TMT 1	Ozone	$\bar{X}$	99.7	332.7	19.6	18.5
		SE	2.2	9.4	1.4	1.6
	No-Ozone	$\bar{X}$	99.0	313.9	18.5	19.4
		SE	2.4	9.4	1.5	2.4
TMT 2	Ozone	$\bar{X}$	98.9	327.9	19.1	18.7
		SE	2.1	10.4	1.5	1.8
	No-Ozone	$\bar{X}$	98.6	312.0	19.6	17.4
		SE	2.2	10.6	1.5	1.7
TMT 3	Ozone	$\bar{X}$	115.3	521.4	21.9	25.5
		SE	2.3	15.5	1.5	2.1
	No-Ozone	$\bar{X}$	114.6	508.9	20.9	26.4
		SE	2.2	13.9	1.6	2.6

All symbols have been defined previously in Table 1, Table 4, or the text.

TABLE 7. Treadmill Data (Females - Study 2)

			HR (bpm)	$\dot{V}_E$ /kg (mL/min/kg)	(rpm)	$V_T$ /kg (mL/kg)
TMT 1	Ozone	$\bar{X}$	104.9	264.4	18.8	14.2
		SE	4.0	11.7	0.9	0.6
	No-Ozone	$\bar{X}$	101.7	248.3	18.4	13.9
		SE	3.2	10.2	0.9	0.6
TMT 2	Ozone	$\bar{X}$	99.3	248.3	20.1	12.7
		SE	2.6	13.0	1.0	0.7
	No-Ozone	$\bar{X}$	98.6	253.3	19.7	12.9
		SE	3.2	11.9	1.0	0.6
TMT 3	Ozone	$\bar{X}$	109.6	336.1	22.1	15.3
		SE	3.0	17.1	0.9	0.7
	No-Ozone	$\bar{X}$	110.8	342.1	21.5	16.1
		SE	3.8	16.1	0.8	0.6

All symbols have been defined previously in Table 1, Table 4, or the text.

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## SECTION V

### ASSESSMENT OF THE EFFECTS OF OZONE ON COMPONENTS OF THE BLOOD

J. M. McKenzie

#### Introduction.

In the blood, as in other biological systems, the deleterious effects of ozone are most probably mediated through the oxidation and peroxidation of labile chemical bonds, such as those found in the sulfhydryl-containing enzymes and the double bonds of unsaturated lipids. For this reason several of these enzymes were measured in these experiments, as were the aldehyde peroxidation products. There were also included some tests that are less well defined with respect to chemical specificity but which, nevertheless, are useful in detecting gross structural and metabolic changes due to ozone damage. Moreover, because ozone's effects on the blood in vivo are known to be enhanced by increases in pulmonary ventilation, this factor was introduced by requiring the subjects of the first two studies (exposure levels of 0.20 and 0.30 ppmv, respectively) to perform mild exercise during the exposure period.

In experiments involving the use of naive subjects one must always consider the probability of emotional stress due to unfamiliar laboratory surroundings. This stress can influence experimental results over and above any effects of the environmental factor under investigation. An effective way of controlling such nonspecific stress is to vary equally the order of presentation, so that half of the subjects are first presented with the control condition, while the other half experience the experimental condition on their first visit to the laboratory. This tactic was used in the present studies. Additionally, in order to evaluate the stress due to ozone per se, the urine that formed during the control and exposure periods was collected and analyzed for the stress indicator hormones, epinephrine, norepinephrine, and the 17-ketogenic steroids.

#### Methods.

Within 10 min after completion of both ozone and no-ozone periods, approximately 10 mL of blood were withdrawn from the antecubital vein into new plastic syringes. Blood samples were immediately mixed with heparin and small portions were taken for microhematocrit measurements and microscopic study. Approximately 2 mL of blood were placed immediately into a sterile 13- by 100-mm glass tube which was covered with a sterile cotton pledget and placed into a water bath at 37° C for an incubation period of 22 h. Another 2-mL portion was used for immediate (within 1 h) measurement of erythrocyte osmotic fragility.

The remaining blood, about 7 mL, was placed into a conical tube and centrifuged for 15 min to separate the cells and plasma. The plasma was divided into three screw-capped tubes and frozen immediately for later measurements of cholinesterase activity. The buffy coat was discarded and the erythrocyte fraction was washed twice in unbuffered (pH 6) saline; remnants of the buffy coat (and some erythrocytes) were removed after each washing. Erythrocytes were then reconstituted with saline to a hematocrit of approximately 50 percent, separated into three portions, and frozen in screw-capped tubes for later measurements of cholinesterase, hemoglobin, and lipid peroxidation products.

Erythrocyte osmotic fragility was measured twice in each sample: immediately after blood sampling and again after incubating the whole blood sample under aseptic conditions for 22 h at 37° C. The technique is based on a standard clinical method described by Wintrobe (1). Fifty-microliter portions of whole blood were diluted to 5 mL with phosphate-buffered (sodium salts) NaCl solution at pH 7.4. By varying the NaCl concentration a range of osmotic activities was obtained. In these studies the concentrations (equivalent to pure NaCl solution) used were selected to give the best estimates of endpoint; the range was 100 to 850 mg NaCl per dL. Concentrations differed somewhat in Studies 1 and 2, but the range did not vary. After 20 min of incubation at room temperature, 5 mL of 2.0 percent NaCl were added to prevent further hemolysis, the remaining suspended cells were removed by centrifugation, and the hemoglobin that had been released into the supernatant fluid was estimated by measuring the optical density at 540 nm. Readings obtained from the tubes containing 0.85 percent NaCl were subtracted from the data obtained from all other tubes, and the results were divided by those from an average reading of two tubes containing whole blood and deionized H<sub>2</sub>O (100 percent hemolysis) to obtain estimates of percent hemolysis.

In order to evaluate the results of osmotic fragility tests the raw data (percent hemolysis) were converted to ordinate/abscissa values of percent hemolysis vs. mg NaCl/dL. A graphic display of these data reveals a sigmoid progression of hemolysis as the concentration of NaCl is decreased. These data were further reduced to forms suitable for statistical analysis by calculating a sigmoid location parameter (LP) and a scale parameter (SP):

$$LP = \sum_{100}^{850} (C_{i+1} + C_i)/2 (H_{i+1} - H_i)$$

$$SP = \sqrt{\sum_{100}^{850} ((C_{i+1} + C_i)/2)^2 (H_{i+1} - H_i) - (LP)^2}$$

where C and H are specific concentrations used and percent hemolysis, and the data are taken over a concentration range of 100 to 850 mg NaCl per dL.



These sigmoid parameters, LP and SP, are analogous to the mean and standard deviation of frequency distribution graphs. They are independent of one another in normally distributed populations; however, technical barriers prevent verification of normal distributions in these data.

Hemoglobin concentration was the basis for expressing the results of other assays performed on the washed erythrocyte fraction. Hemoglobin was measured by an automated adaptation of the cyanomethemoglobin described by Wintrobe (1).

Reticulocytes were counted in blood smears after staining with brilliant cresyl blue. Two slides were prepared from each sample. In each slide 1,000 cells were examined in contiguous fields and the frequency of reticulocytes was recorded and averaged for the two slides.

Enzyme activities were assayed by standard methods. Cholinesterase was measured by an automated adaptation of Ellman's method (2); in the automated procedure described by Fowler and McKenzie (3) the thawed erythrocytes are prediluted 1/50 with deionized H<sub>2</sub>O, while the plasma samples are prediluted with 0.85 percent NaCl<sup>2</sup> solution. In samples of whole blood taken in Study 2 (0.30-ppmv exposure) the enzymes glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) were measured in the laboratory of Dr. B. Connor Johnson at the University of Oklahoma Health Sciences Center, Oklahoma City. Standard spectrophotometric recording methods were used.

Lipid peroxidation products were measured by the thiobarbituric acid (TBA) method (see Mengel (4) for references). One-half milliliter of the erythrocyte fraction was mixed (vortex mixer) in a conical centrifuge tube with 1.5 mL of 10.0 percent aqueous trichloroacetic acid (TCA) solution. After centrifuging, 1.0 mL of the supernatant solution was removed and mixed with 1.2 mL of aqueous TBA; the mixture was heated for 15 min in a boiling water bath, then cooled. Optical density was measured in a Ziess PMQ-II spectrophotometer at 535 nm. The blank consisted of a 1.0/1.2 mixture of TCA/TBA and the instrument was calibrated with solutions (TCA/TBA) of malonaldehyde derived from (1,1,3,3-tetramethoxypropane malonaldehyde bis-(dimethyl acetal); Aldrich No. 10,838-3). The method described here is reproducible; recoveries of 0.688, 1.375, 2.750, and 5.000 nmol malonaldehyde from a supernatant solution of blood/TCA ranged from 88 to 102 (average 91) percent. There was no correlation between percent recovery and the amount of malonaldehyde added.

Stress indicator hormones were measured by techniques used in our laboratory for the past 7 years (5). Immediately following the periods of exposure to ozone and following the control experiments the urine that had formed during the period was collected in tared

plastic bottles containing an excess of boric acid and chilled immediately. Within 3 h, portions were placed into vials and frozen for later assays of catecholamines and the 17-ketogenic steroids. Those aliquots kept for analysis of catecholamines were made 0.05N for HCl before freezing. After measuring the stress indicators the concentration data were corrected for the total weight of urine collected and for the duration of the collection period, so that the results could be expressed in terms of micrograms of metabolite per hour of collection.

Statistical analyses were made using standard methods. When data could be considered as paired (e.g., control and ozone exposure data from individual subjects), the paired t-test was used. Otherwise, unpaired tests were made. Degrees of freedom varied because of missing data.

### Results.

Erythrocyte osmotic fragility was not significantly affected by exposure to ozone. The results of tests using fresh blood are shown in Tables 1-3. These parametric data represent the overall response of an erythrocyte population to increasing degree (decreasing NaCl concentration) of osmotic challenge. Parameter LP represents a central point, the concentration at which 50 percent of the cells are hemolyzed; SP represents the "spread" of the data: an increase in SP indicates a wider range in the osmotic resistance of the cell population. Increases in LP and SP can be interpreted as signs of decreased osmotic resistance. Both these parameters were significantly increased by storage at 37° C overnight, but the effect was equal in samples taken after ozone and control exposures. These data are not shown in the tables, because they have no bearing on the question of ozone toxicity. Compared to simple storage effects, the effects of ozone are neither so profound nor so significant. For example, storage resulted in an increase of approximately 20 percent in the LP, whereas the results presented in Table 1 indicate only a small, albeit significant, effect in males. This was shown by statistical analysis to be due to an order effect; in our analyses the data after ozone exposure were always compared to those derived from control experiments. Thus, ozone appears to have an effect only if, for these naive subjects, ozone is presented on their first visit to the laboratory. This influence is shown more clearly in the results of Study 2 which demonstrate a significant effect of order in the combined male and female groups. This was also found in blood kept for 22 h at 37° C. There was no significant influence either of ozone or of order on the SP parameter (Table 2).

Table 3 is included here to demonstrate the precision of LP and SP measurements over time. These data are from a single male subject who did not participate in the exposure experiments but who contributed samples of blood each week for the 8-week duration of Study 1.

Hematocrit (Table 4) was not affected by exposures to ozone; however, in Study 1 there was a significant effect of order. Hematocrit tended to be higher on the first visit to the laboratory.

Reticulocyte frequency data showed no changes due either to ozone or to order. The frequency ranges were 0.1-1.5 for male subjects and 0.1-2.7 per 100 erythrocytes for female subjects.

Enzymes were unaffected by exposure to ozone. With the exception of a significant order effect on 6PGD in the female subjects of Study 2 there were no signs of changes in enzyme levels.

Lipid peroxidation products (Table 8) were essentially at the same levels in all samples. That there were measurable quantities of these products is probably attributable to the sensitivity of the test and/or to slight oxidation that may have occurred during preparation of the samples.

Stress indicator hormones\* exhibited differences in excretion patterns that were entirely a function of presentation order. This is most apparent in Table 9 which shows that the excretion rate for epinephrine was always greater after the first experiment, regardless of the conditions of that experiment. These differences between the first and second visits to the laboratory are reflected in statistically significant differences between control and ozone exposure values for variables 1, 3, and 4 for Study 1, and by a highly significant effect of order in that study. No changes were apparent in norepinephrine excretion rates (Table 10), but the order effect is the only significant influence apparent in Table 11, which presents the data for excretion rates of the 17-ketogenic steroids. No influence of ozone on these hormones could be detected.

#### Discussion.

Ozone is a highly reactive compound; much that is inhaled is broken down in the respiratory tract before it can reach the blood-alveolar interface. For this reason the threshold for damage by ozone to the blood elements cannot be predicted by in vitro experiments

\*A comment on the order effect, seen in some of these data, is appropriate: Measurement of the stress indicator hormones provided a means of detecting the stress that obtained when subjects visited the laboratory for the first time. The correspondence between increases in the excretion of these hormones and changes in some of the hematological variables should not be interpreted, however, as reflecting any causal relationships between them.

TABLE 1. Effects of Exposure to Ozone on Osmotic Fragility (LP) of Erythrocytes. Fresh Blood.

Units: mg NaCl per dL ( $\pm$  Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	411.5(10.0)	414.3 (8.4)	7	427.9(16.4)	428.4(15.5)
2	6	403.3(16.5)	405.7(12.7)	7	425.7(10.4)	423.4 (8.5)
3	5	400.8 (5.0)	411.0 (7.6)	7	425.4(15.1)	427.3(15.2)
4	8	407.0 (8.3)	406.5 (8.3)	7	418.7 (9.1)	415.0(12.9)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

<u>VARIABLE</u>	<u>P</u>	<u>STATISTIC</u>
Ozone Effect	Study Number 1	Study Number 2
1	NS	NS
2	NS	NS
3	<0.05	NS
4	NS	NS
Sex Effect	NS	NS
Order Effect	NS	<0.05

NS: Not significant

TABLE 2. Effects of Exposure to Ozone on Osmotic Fragility (SP) of Erythrocytes. Fresh Blood.

Units: mg NaCl per dL (+ Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	57.7(10.2)	64.0(18.0)	7	56.4(4.2)	56.6(3.7)
2	6	80.0 (8.0)	67.7(13.8)	7	58.6(3.3)	58.9(2.3)
3	5	66.2(20.9)	50.8(10.6)	7	55.1(4.9)	52.9(1.1)
4	8	75.9 (7.0)	72.1 (9.2)	7	56.0(3.1)	54.9(3.8)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

<u>VARIABLE</u>	<u>P</u>		<u>STATISTIC</u>
Ozone Effect	Study Number 1		Study Number 2
1	NS		NS
2	NS		NS
3	NS		NS
4	NS		NS
Sex Effect	NS		NS
Order Effect	NS		NS

TABLE 3. Control Osmotic Fragility Data.  
Sigmoid Location (LP) and Scale (SP) Parameters  
Over the 8-Week Experiment.\*

	LP		SP	
	FRESH BLOOD	STORED BLOOD	FRESH BLOOD	STORED BLOOD
	409	487	45	98
	416	472	57	93
	416	492	61	93
	400	474	78	105
	403	462	76	103
	406	470	60	102
	420	491	55	91
	<u>399</u>	<u>480</u>	<u>79</u>	<u>92</u>
MEAN	408.6	478.5	63.9	97.1
SD	8.0	10.8	12.4	5.6

\*See text for details.

TABLE 4. Effects of Exposure to Ozone on Hematocrit

Units: Percent Hematocrit ( $\pm$  Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	41.5(2.4)	41.0(1.5)	7	42.4(1.5)	41.9(2.4)
2	6	41.5(2.2)	42.6(1.7)	7	39.9(3.1)	40.8(3.2)
3	7	47.6(2.0)	47.0(2.2)	7	45.9(1.5)	45.9(1.6)
4	7	46.7(3.2)	47.1(2.7)	7	45.2(2.0)	45.8(1.9)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

VARIABLE	P	STATISTIC
	Study Number 1	Study Number 2
Ozone Effect		
1	NS	NS
2	NS	NS
3	NS	NS
4	NS	NS
Sex Effect	NS	NS
Order Effect	<0.05	NS

TABLE 5. Effects of Exposure to Ozone on Acetylcholine Esterase Levels of Erythrocytes

Units: Relative Enzyme Units per g  
of Hemoglobin (± Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	.367(.042)	.387(.052)	7	.241(.015)	.241(.021)
2	5	.374(.055)	.374(.065)	7	.251(.033)	.251(.025)
3	7	.385(.063)	.384(.066)	7	.236(.028)	.239(.026)
4	7	.379(.030)	.381(.034)	7	.233(.030)	.237(.037)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

<u>VARIABLE</u>	<u>P</u>	<u>STATISTIC</u>
Ozone Effect	Study Number 1	Study Number 2
1	NS	NS
2	NS	NS
3	NS	NS
4	NS	NS
Sex Effect	NS	NS
Order Effect	NS	NS



TABLE 6. Effects of Exposure to Ozone on Acetylcholine  
Esterase Levels of Plasma

Units: Relative Enzyme Units per mL  
of Plasma (+ Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	31.58 (7.98)	29.68 (8.31)	7	25.66(5.17)	25.37(4.91)
2	5	26.78 (4.24)	27.87 (4.30)	7	24.10(4.70)	24.26(4.72)
3	7	48.55(11.77)	47.95(13.03)	7	33.21(5.44)	33.08(5.58)
4	7	37.13 (8.53)	37.29 (8.44)	7	33.61(5.30)	33.64(5.19)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

<u>VARIABLE</u>	<u>P</u>	<u>STATISTIC</u>
Ozone Effect	Study Number 1	Study Number 2
1	NS	NS
2	NS	NS
3	NS	NS
4	NS	NS
Sex Effect	NS	NS
Order Effect	NS	NS

TABLE 7. Effects of Exposure to 0.30 ppmv Ozone on Levels of G6PD and 6-PGD in Erythrocytes

Units: Enzyme Units per mL  
of Whole Blood ( $\pm$  Standard Deviation)

VARIABLE*	Glucose-6-Phosphate Dehydrogenase			6-Phosphogluconate Dehydrogenase		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	7	2.27(0.52)	2.43(0.43)	7	.623(.338)	.590(.306)
2	7	2.26(0.34)	2.53(0.63)	7	.773(.228)	.533(.255)
3	6	2.03(0.40)	1.97(0.29)	6	.633(.201)	.627(.227)
4	7	2.16(0.56)	2.04(0.43)	7	.514(.236)	.647(.233)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

VARIABLE	P	STATISTIC
Ozone Effect	G6PD	6PGD
1	NS	NS
2	NS	NS
3	NS	NS
4	NS	<0.05
Sex Effect	NS	NS
Order Effect	NS	NS

TABLE 8. Effects of Exposure to Ozone on Levels of Lipid Peroxidation Products in Erythrocytes

Units: Equivalent to nmols of Malonaldehyde per g of Hemoglobin (± Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	23.45 (6.17)	31.14(13.89)	7	38.84(15.28)	36.21(15.37)
2	5	44.40(28.40)	29.40 (9.71)	7	24.73 (8.04)	26.41 (6.84)
3	7	20.46 (4.28)	29.02 (6.17)	7	34.74(10.15)	37.96(17.14)
4	7	24.66 (8.37)	28.25(14.44)	7	39.87 (8.95)	33.53(11.39)

\*- KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

VARIABLE	P		STATISTIC	
	Study Number 1		Study Number 2	
Ozone Effect				
1	NS		NS	
2	NS		NS	
3	NS		NS	
4	NS		NS	
Sex Effect	NS		NS	
Order Effect	NS		NS	

TABLE 9. Effects of Exposure to Ozone on  
Urinary Excretion of Epinephrine

Units: Micrograms Excreted per Hour ( $\pm$  Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	.331(.124)	.313(.119)	7	.333(.098)	.302(.086)
2	6	.324(.119)	.372(.133)	7	.231(.080)	.241(.112)
3	7	.489(.133)	.394(.094)	7	.390(.114)	.382(.105)
4	8	.404(.126)	.502(.100)	7	.356(.107)	.412(.139)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

<u>VARIABLE</u>	<u>P</u>	<u>STATISTIC</u>
<u>Ozone Effect</u>	<u>Study Number 1</u>	<u>Study Number 2</u>
1	<0.05	NS
2	NS	NS
3	<0.01	NS
4	<0.05	NS
<b>Sex Effect</b>	NS	NS
<b>Order Effect</b>	<0.01	NS

TABLE 10. Effects of Exposure to Ozone on  
Urinary Excretion of Norepinephrine

Units: Micrograms Excreted per Hour ( $\pm$  Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	.560 (.198)	.577 (.181)	7	1.258 (.509)	1.335 (.701)
2	6	.857 (.557)	.811 (.294)	7	1.830(1.980)	1.444(1.104)
3	7	1.004 (.429)	.987 (.314)	7	1.647 (.821)	1.449 (.608)
4	8	.929 (.413)	.890 (.330)	7	1.230 (.535)	1.437 (.517)

\*-KEY: 1-Female; control first; 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

VARIABLE	P	STATISTIC
	Study Number 1	Study Number 2
Ozone Effect		
1	NS	NS
2	NS	NS
3	NS	NS
4	NS	NS
Sex Effect	NS	NS
Order Effect	NS	NS

TABLE 11. Effects of Exposure to Ozone on  
Urinary Excretion of 17-Ketogenic Steroids

Units: Micrograms per Hour ( $\pm$  Standard Deviation)

VARIABLE*	Study Number 1; 0.20 ppmv			Study Number 2; 0.30 ppmv		
	N	CONTROL	OZONE	N	CONTROL	OZONE
1	6	308 (78)	291 (78)	7	428(104)	436(119)
2	6	358 (68)	336 (68)	7	404(224)	437(174)
3	7	488(147)	421(134)	7	704(253)	704 (69)
4	8	508(155)	621(150)	7	643(222)	667(139)

\*-KEY: 1-Female; control first. 2-Female; ozone first.  
3-Male; control first. 4-Male; ozone first.

STATISTICAL ANALYSES: Ozone Effect.

<u>VARIABLE</u>	<u>P</u>	<u>STATISTIC</u>
Ozone Effect	Study Number 1	Study Number 2
1	NS	NS
2	NS	NS
3	NS	NS
4	NS	NS
Sex Effect	NS	NS
Order Effect	<0.05	NS

in which isolated whole blood is directly exposed to mixtures of the gas. Predictability of threshold is also confounded by the fact that it is influenced by the rate of pulmonary ventilation, possibly because the ventilation rate determines the fraction of inspired ozone that reaches the blood. This is an important consideration in designing studies for evaluating the hazards of ozone and is behind the rationale for including treadmill exercise in Studies 1 and 2. Because the effects of ozone are enhanced by the rapid breathing that occurs during exercise, the results of these two studies are most applicable to the flight attendant, who is required to perform physical work during those portions of the flight when ozone is most likely to appear. The level of exercise included in Studies 1 and 2 approximated this work.

Except for responses caused by the stress of an unusual situation, none of our subjects exhibited any changes in hematology or in the excretion of stress indicator hormones during these experiments. It may therefore be concluded that, below an ozone level of 0.30 ppmv, there is little hazard to the population of flight attendants from single 3-h exposures to ozone. Furthermore, because passengers are subject to less physical exertion and are less likely to hyperventilate, it may be surmised that the level of 0.30 ppmv is even further below threshold for passengers of comparable age and state of health. These qualifications are important, however: The results of the present studies offer no assurance that people with certain medical conditions, degrees of hypovitaminosis, or inherited enzyme deficiencies, for example, cannot be unusually susceptible to the deleterious effects of ozone. Also, it is more likely that these conditions will occur in the passenger group, whose age and medical status are beyond the control or even the knowledge of the airlines or the FAA.

Other investigators have shown that oxygen in a variety of its chemical forms can damage the blood. Buckley et al. (6), for example, reported lipid peroxidation products and decrements in some of the sulfhydryl-containing enzymes following exposures of human subjects to ozone at 0.50 ppmv, and Mengel and Kann (4) found lipid peroxidation and hemolysis in mice after 1.5-h exposures to 100 percent oxygen at 60 psi (4 atm). Similar damage to erythrocytes by peroxide is the basis for clinical tests of erythrocyte stability. Thus, there can be little doubt that oxidative damage to the blood is possible. Furthermore, a number of conditions that render the blood more susceptible to this damage are known to exist. The enzyme G6PD, for example, serves to protect erythrocytes from oxidative damage (7), and inherited deficiencies of this enzyme occur with significant frequency in certain black and mediterranean populations (8). It has been proposed on these and on theoretical grounds that ozone may be particularly hazardous to individuals who are deficient in this enzyme.

Another inherited abnormality that may be present to a significant degree in the passenger population is hemoglobin-S, the blood pigment

responsible for sickle cell anemia in those who are homozygous for the sickle gene. Others who are genetic carriers of the disease, whose erythrocytes contain less than 50 percent hemoglobin-S, are free of the disease and most of its signs. Recently, however, it has been demonstrated that hemoglobin-S, in addition to its tendency to polymerize under hypoxic conditions, is also much more susceptible to denaturation when oxygen tensions are high (9). This effect may be associated with low body levels of vitamin E (10). The protective effects of vitamin E may be due to its influence on glutathione peroxidase, another of the enzymes that protect against oxidative damage. Chiu and Lubin (11), studying a group of patients with sickle cell disease, found levels of this enzyme to be negatively correlated with those of vitamin E. This observation is consistent with a hypothesis that levels of the peroxide-destroying enzymes may be influenced by levels of their substrate. Vitamin E, which is not synthesized in the human body, may serve as a rapidly acting scavenger of peroxides, removing them before they are able to exert this influence.

Regardless of the specific mechanisms involved in protecting the blood against ozone, it is highly probable that this protection may not be equally available to the variety of people who travel by air. The magnitude of risk to the passenger population cannot be fully appraised without additional investigations especially designed to evaluate factors and combinations of factors, such as repetition of exposure, age, and the various suspect medical and genetic conditions. Until such inquiries are completed it might be wise to limit the frequency of exposure, even in healthy, young people, and to seek medical consultation for those individual cases in which an unusual susceptibility seems likely. Moderate doses of the anti-oxidant vitamins E and C might afford some protection against ozone, but there is no hard evidence that these vitamins have any value when there is no deficiency. Certainly, any reliance on such use for protection against levels of ozone known to be dangerous would be very foolhardy indeed.



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## SECTION VI

### THE EFFECTS OF OZONE ON PHOTOPIC, MESOPIC, AND SCOTOPIC VISION

John A. Vaughan

#### Introduction.

The assessment of the possibility of impaired vision attributed to the exposure of human test subjects to ozone was determined by a battery of 12 vision tests conducted when the test subjects were light adapted (photopic vision), twilight or semidark adapted (mesopic vision), or dark adapted (scotopic vision). The following tests were given for each of the above illumination categories: Photopic vision included tests for distant visual acuity, stereoscopic acuity, vertical and lateral phorias, accommodation, hand-eye coordination, color vision, and blink rate; mesopic vision tests consisted of determinations of central visual fields, and retinal bleach recovery; and scotopic vision was assessed by tests of numerical recognition and dark adaptation.

The purpose of this experiment then, was to compare the effects on vision in room air (no-ozone) with conditions of 0.20 ppmv ozone with exercise (Study 1), 0.30 ppmv ozone with exercise (Study 2), and 0.30 ppmv without exercise (Study 3).

#### Methods.

The test subjects were examined for distant visual acuity, near visual acuity, and stereoscopic acuity before being selected. Subjects wore corrective lenses when required for good vision throughout the two exposure periods. In Study 1, 14 subjects (nine males and five females) wore no prescription lenses, 11 (five males and six females) wore prescription spectacle lenses, and 2 subjects (one male and one female) wore contact lenses. In Study 2, of the 28 subjects tested, 16 had no lens correction (eight males and eight females), 6 (four males and two females) wore prescription spectacle lenses, and 6 (two males and four females) wore contact lenses. In Study 3, of the 28 subjects tested, 13 (seven males and six females) had no corrective lenses, 11 (six males and five females) wore prescription spectacle lenses, and 4 (one male and three females) wore contact lenses.

#### Photopic Vision.

The Titmus Vision Tester was employed to determine the parameters of distant visual acuity for each eye (slides ARF-1 and ALF-1),

stereoscopic acuity (slide SDF-1), vertical phoria (slide VPF-1), and lateral phoria (slide LPF-1). Accommodation was measured with the Royal Air Force Near Point Rule, using techniques of "out-to-clear" and "in-to-blur." Color vision was determined with the Farnsworth Panel D-15 Color Test. The hand-eye coordination task consisted of an "Etch-a-Sketch" with a design consisting of two parallel lines drawn on a clear plastic overlay. The subject was required to "draw" a line between the limiting lines on the overlay. The procedure was timed with a stopwatch and errors were recorded on a Polaroid photograph. Measurements of blink rate were made by an outside observer looking into the altitude chamber through a one-way glass temporarily positioned over the window. One observer counted the eyeblinks with a laboratory counter, while a second observer timed the procedure for 1 min with a stopwatch. Four 1-min counts were made by the same observer on each subject; means and ranges were calculated later.

#### Mesopic Vision.

The bleaching recovery test was conducted using the numbers with the visual acuity fraction of 20/61 (2.5-mm height) on the slide. The observer occluded the test numbers and set the test light at 5.5 (on a scale of 7.0) log units, and the test subject again positioned his head on the chinrest. The bleaching lights were turned on for 3 min; immediately following the bleach, the observer simultaneously removed the occluder and started a stopwatch. Bleach recovery time was the number of seconds required for the subject to read the numbers correctly.

Central visual fields were determined under mesopic vision conditions (chamber lights out but windows uncovered) with the Harrington-Flocks Multiple Pattern Visual Field Screener (Model B-11). Errors were recorded for 10 presentations to each eye.

#### Scotopic Vision - Dark Adaptation.

After the subjects were given a final briefing, the overhead lights were extinguished. Each subject positioned his head on the chinrest of the light chamber of the Goldmann-Weekers Adaptometer (Haag-Streit) with the test aperture occluded, and two 60W bleaching lights reflecting 700 fL were turned on for a period of 3 min. Immediately after the lights were turned off, the subject donned a pair of opaque goggles for the next 25 min. The subject then took off the goggles and repositioned his/her head on the chinrest. For each scotopic vision test, a black velvet cloth was draped around the subject's head and shoulders to exclude any ambient light. The subject was asked to gaze at a dim, red fixation light about  $11^{\circ}$  above the test plate mounted in the aperture. The test plate, 5.5 cm in diameter, consisted of alternating black and white bars (100 percent contrast). The black bars were opaque but the translucent white bars transmitted 49 percent of the light and were blacklighted by a 9W test lamp. The

intensity was controlled by the observer, from 0 to 10 mL as calibrated with the Pritchard Spectra Photometer, Model 1970-PR. The illumination intensity of the test light was synchronized with a mechanically linked recording arm and stylus that traveled vertically along the ordinate of a chart attached to a rotating drum. With the test light turned to its lowest position (no light), the observer signaled "read," and slowly increased the illumination with the test light. The test subject tapped the instant he/she could identify the orientation of the bars (horizontal, vertical, or oblique), and the observer perforated the chart with the stylus at that point. The observer then changed the orientation of the bars and the procedure was repeated twice more within the 2-min period. The subject was then allowed to remove his/her head from the apparatus for 6 min, and three more consecutive measurements were taken in a second 2-min period. This modified procedure replaced the usual method of measuring dark adaptation (taking a point at 1- or 2-min intervals) because trial runs demonstrated a large decrease in ozone concentration breathed by the subject with his/her head in the adaptometer during the first 30 min, whereas ozone concentration decrements were small during the two 2-min test periods.

During the numerical recognition test, the subject maintained scotopic vision while the observer removed the bar test plate and positioned a plastic visual acuity slide on the adaptometer. The slide contained six groups of numbers in graded sizes, of which only four groups were used. Each group contained three 2-digit numbers. Heights of the numbers were 1.0 mm, 1.5 mm, 2.5 mm, and 4.5 mm, with visual acuity equivalents of 20/25, 20/37, 20/61, and 20/105, respectively, after correction for viewing distance of 30 cm recommended for the adaptometer (1). With the occluder in place, the test light at the lowest position and the subject's head on the chinrest, the observer removed the occluder and slowly increased the target illumination until the subject again signaled "stop," and identified the six numbers. As before, the subject repeated the procedure until the four groups of numbers were read.

Statistical analyses consisted of paired t-tests for all visual parameters except the numerical recognition test which employed an analysis of variance with a randomized block factorial design. Means of the paired data for no-ozone vs. ozone were treated separately for males, females, and for males and females combined. Unpaired t-tests were used to determine differences between the male and female test subjects (2).

## Results.

### Photopic Vision.

Visual acuity. The effects of ozone on distant visual acuity, in terms of the Snellen fraction, are presented in Table 1. Although there were no significant differences in visual acuity with ozone in Studies 1 and 2, combined subjects in Study 3 showed significant impairment of visual acuity during exposure to ozone.

A total of 46 test subjects (55.4 percent) maintained distant visual acuities of 20/20 or better (20/13 to 20/20) for the means of the left and right eyes during exposure to ozone. Twenty-four were male, and 22 were female. There was a significant loss of acuity in the no-ozone females of Study 1 ( $p \leq .05$ ) and in the males and females of Study 3 ( $p \leq .05$ ) as shown in Table 1. Data of Study 2 showed no changes with ozone.

Stereoscopic acuity. The data for stereoscopic acuity demonstrated no significant changes with ozone (Table 2). The high variability may be explained, in part, by the tendency of the apparatus to cause convergence in some of the test subjects. During preexposure examination, these subjects were tested for stereopsis with the Titmus book test, which contained characters identical with the Titmus Vision Tester. There were no significant differences between males and females.

Vertical and lateral phorias. The numbers and percentages of test subjects who showed the effects of vertical and lateral phorias in an ozone environment are presented in Table 3. Values of vertical phoria ranged from 0.00 to 1.00 Prism Diopters (PD). In the total sample of 83 test subjects of the three studies, 55 (66.3 percent) had orthophoria for both no-ozone and ozone conditions, 22 (26.5 percent) had left hyperphoria, and 6 (7.2 percent) showed right hyperphoria in one or both conditions.

The range of values for lateral phorias was 0.00 to 7.00 PD. Eleven subjects (13.3 percent) experienced orthophoria for both no-ozone and ozone conditions, 61 (73.4 percent) showed some degree of exophoria, and 11 (13.3 percent) had esophoria in one or both conditions of no-ozone or ozone. There were no statistically significant differences in either vertical phorias or lateral phorias between no-ozone and ozone conditions or between males and females.

Accommodation. Mean values of accommodation for both eyes, measured separately with the R.A.F. Near Point Rule are shown in Table 4. Values of individual eyes for the three studies in the "out-to-clear" procedure ranged from 6.0 to 21.5 cm, and for the "in-to-blur" technique ranged from 6.0 to 21.0 cm. Although there were no significant changes with ozone in any of the three studies, values of Study 3 indicated that accommodation in the male subjects was significantly less effective ( $p \leq .05$ ) in the no-ozone condition and using the "out-to-clear" procedure than in the females. Values for females were also significantly higher ( $p \leq .01$ ) than for males for both the no-ozone and ozone conditions using the "in-to-blur" procedure. Except for these significant sex differences, the data indicate that exposure to ozone does not alter accommodation.

Farnsworth Panel D-15 color test. The data of Table 5 indicate no significant differences in the time necessary to complete the color test between the conditions of no-ozone and ozone. Study 1 and Study 2 showed that, although completion times were longer for males they were not significantly different from those for females. However, in Study 3, completion times for the males were significantly longer than for the females at both the no-ozone ( $p \leq .01$ ) and the ozone ( $p \leq .05$ ) conditions. Individual values ranged from 16 s to 136 s for all of the subjects.

Hand-eye coordination. Completion times and errors using the "Etch-a-Sketch" device are listed in Table 7. The number of seconds needed to complete the test ranged from 32 to 209, and the number of errors made ranged from 0 to 10 for the sample population. Except for the no-ozone exposure in Study 2, the males completed the test in slightly less time than the females but made about the same number of errors. There were no statistically significant differences in either completion time or number of errors or between male and female test subjects.

Blink rate and range. Changes in mean blink rates and ranges with exposure to ozone are presented in Table 8. Because blinking may be voluntary, individual blink rates for the three studies for an average of four trials covered a wide range from 4.2 blinks per minute (B/min) to 52.8 B/min. Ranges of blink rates varied from 2 to 24 B/min. Although there were no significant differences in blink rates in Studies 1 and 2, the data of Study 3 showed the rate for females, and males and females together, to be significantly higher with ozone ( $p \leq .05$ ). Males did not differ from females.

#### Mesopic Vision.

Central visual fields. Results of the visual fields measured under mesopic conditions with the Harrington-Flocks Screener are shown in Table 6. Although there were no significant changes with ozone with the means of the left and right eyes of each subject, paired data tests on individual eyes revealed that in Study 1, fields of the right eyes of the females were significantly enlarged with ozone ( $p \leq .01$ ). Data of Study 2 showed slight but no significant impairment of visual fields with ozone, and values of Study 3 indicated significantly more errors ( $p \leq .05$ ) and thus more field degradation with ozone in the left eyes of both males and females. The same pattern of errors occurred when the right and left eyes of each subject were averaged, but the result was not statistically significant. Forty right eyes (48.2 percent) and 43 left eyes (51.8 percent) showed no change in visual fields from no-ozone to ozone (Table 6).

Retinal bleach recovery time. The times required for the subjects to correctly identify numbers at 20/60 visual acuity following a 3-min light bleach are shown in Table 11. Recovery times were generally lower for both male and female subjects, but t-tests revealed no statistically significant decreases in the ozone environment or between male and female test subjects. Bleach recovery times ranged widely, from 5 s to 138 s. The levels of ozone concentration of this study apparently do not influence the neurochemical recovery of the bleached retina.

#### Scotopic Vision.

Visual acuity. The values of Table 9 reflect the subjects' responses to light energy after about one-half hour in total darkness and are measures of the level of dark adaptation. Light energy levels did not change significantly from no-ozone to the ozone exposure, nor were there any statistically significant differences between male and female test subjects.

Numerical recognition. The means and standard deviations of the light energy required for the dark-adapted subjects to read four sizes of numerals are presented in Table 10. Analysis of variance showed significant differences among the sizes of the numbers ( $p \leq 0.01$ ) within conditions, but not between no-ozone and ozone or between males and females.

#### Subjective Questionnaire.

During the debriefing session of Study 1, only one test subject (No. 08F) complained of temporary dryness of the eyes during exposure to 0.20 ppmv of ozone. In Study 2, three male subjects (Nos. 08M, 11M, and 13M) and two female subjects (Nos. 04F and 05F) complained of dry eyes (Nos. 08M and 13M) and burning eyes (Nos. 11M, 04F, and 05F) after walking on the treadmill in an environment of 0.30 ppmv of ozone. Subjects No. 04F and 11M also had coughs and substernal discomfort, which cleared up within 3 h after ozone exposure. Subject No. 08M reported throat irritation but no eye problems, and subjects No. 05F and 13M had no symptoms following exposure to ozone.

After exposure to the same concentration of ozone (0.30 ppmv), but without exercise, 10 subjects of Study 3 reported eye irritation. Subject No. 01M reported slight burning (present but not annoying) and watering, No. 02M had slight burning and dryness, No. 04F reported moderate burning (present and annoying) and dryness, Nos. 05M, 10M, and 14M slight burning, Nos. 07F, 14F, and 11M moderate burning, and No. 10F watering eyes. All the symptoms cleared up after the experiment was over. Because no symptoms were reported by these subjects after the no-ozone exposure, eye irritation was probably caused by ozone and not by other factors.

## Discussion.

Statistical analyses revealed few differences in the vision parameters examined in these studies that could be attributed to ozone. Lagerwerff (3) reported an investigation of the effects of ozone on several visual parameters. Although the methods differed, his results agree with those of this study in that photopic visual acuity, stereopsis, vertical phoria, and color vision were not greatly influenced by a 3- or 4-h exposure to 0.30 ppmv of ozone. Measurements of visual acuity in Study 3 (Table 1) were made 75 min after those of Studies 1 and 2, so that the subjects were exposed to ozone for a longer period of time before the visual acuity determinations were made, which may account for the slight but significant impairment in the photopic visual acuity with ozone in Study 3 with the combined male and female sample population. However, Snellen fractions were barely significantly higher with ozone ( $p < .05$ ) and, since all of the remaining test results were not significantly different from no-ozone, this one statistic probably has little practical meaning.

However, Lagerwerff (3) did report a sizable decrease in scotopic and mesopic visual acuity that is not apparent from the data of this study. Adler (4) states that scotopic vision begins at about 1 microlambert ( $\mu L$ ) and is in effect with increasing illumination up to 550  $\mu L$ . The data of Table 9 show that all of the subjects achieved and maintained scotopic vision within 35 min of dark adaptation. The range of mesopic vision is 0.55  $\mu L$  to 550  $\mu L$  (4). Time values of the retinal bleach recovery test with ozone (Table 11) and errors in the central fields determinations (Table 6) conformed to mesopic vision conditions, and neither retinal bleach recovery times nor visual fields were altered by exposure to ozone.

Lagerwerff also found that the number of subjects that showed a net increase in peripheral visual fields was 76.9 percent in an ozone concentration of 0.35 ppmv during a 3-h exposure period. The values in Table 6 suggest that, of the eyes of the test subjects who showed changes in visual fields with ozone, with one exception of the right eyes of the females and right eyes of the males and females of Study 1, a larger percentage were degraded (26.5 percent) than were improved (25.3 percent). Nearly half of the eyes showed no changes in visual fields with ozone as measured by the Harrington-Flocks apparatus. Data from Lagerwerff's study and from this study suggest that ozone has a greater effect on peripheral fields and a lesser effect on central fields. Although Lagerwerff found no differences in vertical phoria with ozone, he reported an increase in lateral phoria, caused by an indirect mechanism affecting all of the oculomotor muscles except the superior and inferior recti. The data of Table 3, although not statistically significant, show that



lateral phorias showed no changes from the no-ozone to the ozone condition in about 50 percent of the test subjects.

Although an increasing number of subjects in the three studies complained of some eye irritation following exposure to ozone, eye symptoms were not severe and cleared up after the end of exposure. Subjective symptoms of irritation to the eyes and respiratory tract have been reported for ozone concentrations in air greater than 1 ppmv, and concentrations of 2.0 to 3.7 ppmv have caused irritation to normal eyes within 6 minutes (5). This subjective data support the significant increase in the blink rates of Study 3 for the combined male and female test subjects (Table 8). In this study, one man and three women wore contact lenses, and had blink rates in no-ozone and ozone somewhat higher than those of the remaining subjects, but none of the subjects with contact lenses complained of eye irritation. Thus, changes in blink rate may also be attributed to factors of dryness (the relative humidity was about 8 percent in these studies), contact lenses, or the level of activity of the subject at the time, and do not necessarily reflect the irritating effects of ozone at these concentrations.

#### Summary.

Values of the vision parameters of photopic visual acuity, stereoscopic acuity, vertical and lateral phorias, and color vision indicated only minor changes, and ozone had no degrading effects on the 83 test subjects exposed to it in the concentrations studied here. Determination of visual fields showed that the fields of about as many subjects were degraded as were improved when exposed to ozone. Statistical analysis of the data also revealed that accommodation, hand-eye coordination, blink rate and range, scotopic and mesopic vision are not altered to any degree during a 3- or 4-h exposure to a concentration of 0.20 or 0.30 ppmv of ozone.

TABLE 1. Photopic Distant Visual Acuity

SUBJECTS	NO-OZONE		OZONE		t
	Snellen Fraction Mean	S.D.	Snellen Fraction Mean	S.D.	
	<u>STUDY 1</u>				
Males	20/22	20/8	20/23	20/9	0.38(NS)
Females	20/36	20/21	20/31	20/16	1.45(NS)
Males and Females	20/28	20/16	20/26	20/13	1.00(NS)
	<u>STUDY 2</u>				
Males	20/24	20/10	20/26	20/18	0.94(NS)
Females	20/22	20/8	20/25	20/14	1.36(NS)
Males and Females	20/23	20/9	20/26	20/16	1.56(NS)
	<u>STUDY 3</u>				
Males	20/22	20/8	20/24	20/14	1.04(NS)
Females	20/19	20/6	20/22	20/10	2.13(NS)
Males and Females	20/21	20/7	20/23	20/12	2.07( $p \leq .05$ )

TABLE 2. Stereoscopic Acuity

SUBJECTS	NO-OZONE		OZONE		t
	Arc Seconds		Arc Seconds		
	Mean	S.D.	Mean	S.D.	
<u>STUDY 1</u>					
Males	27.0	14.8	28.7	18.5	0.49(NS)
Females	49.2	54.0	54.0	50.9	0.20(NS)
Males and Females	36.8	38.4	38.3	37.4	0.45(NS)
<u>STUDY 2</u>					
Males	58.2	100.8	55.4	101.5	1.05(NS)
Females	37.8	47.9	41.8	50.5	1.09(NS)
Males and Females	48.0	78.1	48.6	78.9	0.23(NS)
<u>STUDY 3</u>					
Males	37.5	47.7	32.8	28.7	0.53(NS)
Females	27.8	22.2	29.6	18.8	0.34(NS)
Males and Females	32.7	36.8	31.2	23.9	0.28(NS)

TABLE 3. Vertical and Lateral Phorias From No-Ozone to Ozone Conditions

CHANGE	MALES			FEMALES			MALES AND FEMALES		
	Vertical		Lateral	Vertical		Lateral	Vertical		Lateral
	(n)	(%)		(n)	(%)		(n)	(%)	
Increased Decreased No Change Totals	3	20.0	3	1	8.3	2	4	14.8	5
	1	6.7	2	3	25.0	6	4	14.8	8
	11	73.3	10	8	66.7	4	19	70.4	14
	15	100.0	15	12	100.0	12	27	100.0	27
Increased Decreased No Change Totals	1	7.2	3	1	7.2	3	2	7.2	6
	3	21.4	2	3	21.4	2	6	21.4	4
	10	71.4	9	10	71.4	9	20	71.4	18
	14	100.0	14	14	100.0	14	28	100.0	28
Increased Decreased No Change Totals	0	0	4	1	7.1	4	1	3.6	8
	0	0	3	2	14.3	5	2	7.1	8
	14	100.0	7	11	78.6	5	25	89.3	12
	14	100.0	14	14	100.0	14	28	100.0	28

TABLE 4. Accommodation

SUBJECTS	NO-OZONE		OZONE		t
	OUT-TO-CLEAR				
	cm		cm		
	Mean	S.D.	Mean	S.D.	
<u>STUDY 1</u>					
Males	10.7	+1.4	10.9	+2.3	1.17(NS)
Females	12.3	+2.6	12.4	+3.2	0.22(NS)
Males and Females	11.4	+2.2	11.6	+2.5	0.84(NS)
<u>STUDY 2</u>					
Males	10.7	+2.4	11.0	+2.5	1.00(NS)
Females	11.9	+2.2	12.0	+2.2	0.58(NS)
Males and Females	11.3	+2.3	11.5	+2.4	1.16(NS)
<u>STUDY 3</u>					
Males	10.4	+2.0	10.7	+2.0	0.92(NS)
Females	12.3	+1.9	12.1	+1.5	0.93(NS)
Males and Females	11.3	+2.1	11.4	+1.8	0.25(NS)
IN-TO-BLUR					
<u>STUDY 1</u>					
Males	10.4	+1.9	10.1	+1.4	0.93(NS)
Females	11.1	+2.4	11.1	+2.6	0.30(NS)
Males and Females	10.7	+2.1	10.6	+2.0	0.74(NS)
<u>STUDY 2</u>					
Males	11.4	+3.6	10.8	+2.8	1.50(NS)
Females	11.4	+2.4	11.6	+1.9	0.71(NS)
Males and Females	11.4	+3.0	11.2	+2.4	0.68(NS)
<u>STUDY 3</u>					
Males	9.8	+1.9	9.8	+1.6	0.06(NS)
Females	12.0	+1.8	12.5	+2.6	0.95(NS)
Males and Females	10.9	+2.1	11.1	+2.5	0.77(NS)

TABLE 5. Time to Complete the Farnsworth Panel D-15 Color Test

SUBJECTS	NO-OZONE		OZONE		t
	Seconds		Seconds		
	Mean	S.D.	Mean	S.D.	
	<u>STUDY 1</u>				
Males	49.1	+13.6	54.2	+20.6	1.24(NS)
Females	34.7	+12.2	32.8	+11.0	0.54(NS)
Males and Females	42.7	+14.7	44.7	+19.9	0.73(NS)
	<u>STUDY 2</u>				
Males	44.6	+15.1	41.8	+14.2	0.63(NS)
Females	36.0	+14.0	35.1	+15.9	0.39(NS)
Males and Females	40.3	+14.9	38.4	+15.2	0.75(NS)
	<u>STUDY 3</u>				
Males	52.7	+24.2	53.6	+29.8	0.19(NS)
Females	30.9	+ 8.0	30.9	+11.7	0.00(NS)
Males and Females	41.8	+20.9	42.3	+25.0	0.17(NS)

TABLE 6. Number and Percentage of Test Subjects With Changes From Control in Central Visual Fields During Exposure to Ozone

	MALES				FEMALES				MALES AND FEMALES			
	Right Eye		Left Eye		Right Eye		Left Eye		Right Eye		Left Eye	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
Improved	7	46.7	3	20.0	STUDY 1							
Degraded	3	20.0	3	20.0	5	41.7	3	25.0	12	44.4	6	22.2
No Change	5	33.3	9	60.0	0	0.0	2	16.7	3	11.2	5	18.5
Totals	15	100.0	15	100.0	7	58.3	7	58.3	12	44.4	16	59.3
					12	100.0	12	100.0	27	100.0	27	100.0
85												
Improved	2	14.3	4	28.6	STUDY 2							
Degraded	7	50.0	2	14.3	1	7.1	2	14.3	3	10.7	6	21.4
No Change	5	35.7	8	57.1	6	42.9	7	50.0	13	46.4	9	32.2
Totals	14	100.0	14	100.0	7	50.0	5	35.7	12	42.9	13	46.4
					14	100.0	14	100.0	28	100.0	28	100.0
Improved	1	7.1	3	21.4	STUDY 3							
Degraded	4	28.6	6	42.9	5	35.7	1	7.1	6	21.4	4	14.3
No Change	9	64.3	5	35.7	2	14.3	4	28.6	6	21.4	10	35.7
Totals	14	100.0	14	100.0	7	50.0	9	64.3	16	57.2	14	50.0
					14	100.0	14	100.0	28	100.0	28	100.0

TABLE 7. Completion Time and Errors of Hand-Eye Coordination Test

SUBJECTS	NO-OZONE						OZONE						t
	Time (Sec.)			Errors (No.)			Time (Sec.)			Errors (No.)			
	Mean S.D.			Mean S.D.			Mean S.D.			Mean S.D.			
	Mean	S.D.		Mean	S.D.		Mean	S.D.		Mean	S.D.		
	STUDY 1												
Males	63.4	23.1		2.1	1.5		65.1	24.5		2.3	1.8		0.58(NS)
Females	98.4	53.2		2.3	2.1		89.6	43.6		1.8	2.6		0.92(NS)
Males and Females	79.0	42.4		2.2	1.8		76.0	35.8		2.1	2.1		0.21(NS)
	STUDY 2												
Males	81.2	31.2		2.1	2.9		73.1	32.0		2.3	3.2		0.35(NS)
Females	75.6	27.4		2.4	2.2		77.1	25.8		3.1	2.6		1.03(NS)
Males and Females	78.4	29.0		2.2	2.5		75.1	28.5		2.7	2.9		1.00(NS)
	STUDY 3												
Males	73.4	38.7		1.5	1.8		74.8	42.0		1.6	1.7		0.35(NS)
Females	75.8	47.5		2.6	2.2		78.1	59.1		1.8	1.9		1.35(NS)
Males and Females	74.6	42.5		2.1	2.1		76.5	50.3		1.7	1.8		0.93(NS)



TABLE 8. Blink Rate and Range

SUBJECTS	NO-OZONE				OZONE				t	
	Blink Rate(B/min)		Range(B/min)		Blink Rate(B/min)		Range(B/min)		Rate	Range
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.		
Males Females Males and Females	<u>STUDY 1</u>									
	20.4	12.6	8.0	3.6	20.0	11.0	7.7	4.9	0.26(NS)	0.19(NS)
	19.3	12.7	6.2	3.3	21.0	9.9	4.8	2.9	0.70(NS)	1.01(NS)
	19.9	12.4	7.2	3.5	20.5	10.3	6.4	4.3	0.39(NS)	0.76(NS)
	<u>STUDY 2</u>									
	25.1	12.7	9.4	4.2	25.1	12.3	11.6	4.5	0.05(NS)	1.45(NS)
	18.9	8.8	6.6	3.8	19.6	10.2	8.4	6.0	0.31(NS)	1.18(NS)
	22.0	11.2	8.0	4.2	22.4	11.4	10.0	5.4	0.23(NS)	1.89(NS)
	<u>STUDY 3</u>									
	15.4	11.4	6.8	4.2	17.2	10.0	7.7	4.0	1.02(NS)	0.73(NS)
	18.1	8.4	6.4	3.7	22.4	10.3	7.4	6.0	2.65(p < .05)	0.77(NS)
	16.8	9.9	6.6	3.9	19.8	10.4	7.6	5.0	2.54(p < .05)	1.08(NS)

TABLE 9. Scotopic Vision

SUBJECTS	NO-OZONE		OZONE		t
	$\mu$ L		$\mu$ L		
	Mean	S.D.	Mean	S.D.	
	<u>STUDY 1</u>				
Males	0.30	$\pm 0.20$	0.33	$\pm 0.27$	0.44(NS)
Females	0.68	$\pm 0.61$	0.33	$\pm 0.22$	1.89(NS)
Males and Females	0.47	$\pm 0.47$	0.33	$\pm 0.25$	1.42(NS)
	<u>STUDY 2</u>				
Males	0.58	$\pm 0.32$	0.74	$\pm 0.75$	0.70(NS)
Females	0.57	$\pm 0.33$	0.75	$\pm 0.53$	1.51(NS)
Males and Females	0.58	$\pm 0.32$	0.74	$\pm 0.63$	1.39(NS)
	<u>STUDY 3</u>				
Males	0.46	$\pm 0.28$	0.57	$\pm 0.38$	0.93(NS)
Females	0.63	$\pm 0.54$	0.56	$\pm 0.39$	0.86(NS)
Males and Females	0.54	$\pm 0.43$	0.56	$\pm 0.38$	0.24(NS)



TABLE 11. Retinal Bleach Recovery Time

SUBJECTS	NO-OZONE		OZONE		t
	Time (sec)		Time (sec)		
	Mean	S.D.	Mean	S.D.	
<u>STUDY 1</u>					
Males	22.0	+15.2	26.8	+10.0	1.38(NS)
Females	33.2	+22.7	33.8	+11.8	0.09(NS)
Males and Females	27.0	+19.4	29.9	+11.2	0.78(NS)
<u>STUDY 2</u>					
Males	38.2	+29.6	32.2	+22.2	0.81(NS)
Females	57.5	+46.8	37.9	+22.1	1.39(NS)
Males and Females	48.2	+39.9	35.2	+21.9	1.61(NS)
<u>STUDY 3</u>					
Males	38.2	+23.6	38.3	+17.6	0.03(NS)
Females	46.6	+23.1	41.7	+21.6	0.75(NS)
Males and Females	42.7	+23.3	40.2	+19.5	0.59(NS)

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## SECTION VII

### DISCUSSION AND CONCLUSIONS

C. E. Melton

No significant effects attributable to ozone were demonstrated in the first study. The reason for this appears to be that 0.20 ppmv ( $\sim 400 \mu\text{g}/\text{m}^3$ ) for 4 h at 6,000 ft MSL, even with exercise, is innocuous for normal people. The methods employed appear to be adequately sensitive in that effects related to the order of presentation of the protocol on heart rate, corticosteroid and epinephrine excretion, and RBC fragility were readily shown.

All exercising subjects showed some effects of 0.30 ppmv ( $\sim 600 \mu\text{g}/\text{m}^3$ ) ozone (second study), which means that the threshold for ozone effects lies at some level between 0.30 and 0.20 ppmv. Exercise and oral breathing are clearly aggravating factors in ozone toxicity because sedentary subjects in the third study were unaffected by 0.30 ppmv ( $\sim 600 \mu\text{g}/\text{m}^3$ ).

The effects attributable to ozone conform fairly closely to those reported in the scientific literature and to complaints by flight attendants. These effects are mainly on the respiratory system, the commonest symptom being cough and the most prominent objective effect being restriction of airflow in the bronchioles. The latter finding tells us that, in exercising subjects breathing through the mouth, ozone is penetrating deeply into the lung; in short, is not being entirely scrubbed in the upper respiratory passages. This latter observation points to the obvious desirability of consciously breathing through the nose when ozone levels greater than 0.20 ppmv are encountered. It would be interesting and perhaps of value to know whether or not smoking has a truly mitigating action on the effect of ozone on small airways. The presence of residual smoke in the deep portions of the lung might scrub ozone at the terminal bronchiolar and alveolar levels.

The suggestion in these studies that females are more susceptible than males to adverse effects of ozone should be examined further. Complaint files should be carefully examined for qualitative as well as quantitative differences in the symptoms reported by male and female flight attendants. Further, a debriefing and/or interview program should be instituted for flight deck crews as well as flight attendants to determine the true incidence of complaints. Fewer complaints from flight deck personnel than from cabin personnel may be related to the fact that most pilots and flight engineers are males with relatively sedentary duties, whereas most flight attendants are females and are active in flight.

Extrapulmonary effects, aside from eye irritation, remain difficult to explain. In these studies no effects were shown on the several measurements made on blood. The presence of effects of order of presentation of the experimental conditions tells us that the methods

were probably sensitive enough to pick up effects of ozone had they been present. The presence of an effect on the heart rate of females on their first ozone exposure is most logically explainable by summative anxiety attendant on (i) their first exposure to the experiment (order effect), and (ii) awareness of ozone's odor. Why females should be preferentially affected either by ozone directly or by the above anxiety factors indirectly is not readily explainable. No apparent mechanism can be visualized that would provide for transport of ozone to the heart for a direct effect or to the nervous system for an indirect effect on the heart rate. The demonstration of positive effects on the small airways and lack of effects on blood leads one to theorize that ozone did not penetrate beyond the terminal bronchioles.

Persistence of ozone effects beyond the experimental exposure points to superficial damage to the lining of the respiratory passages that requires time for repair. These persistent effects are readily reversible, however, at the levels of ozone tested. The effects of repeated irritation of this type by ozone are not known.

Retinal or central nervous system effects on vision were of great concern at the inception of this work. Such concerns were aroused by the work of Lagerwerff (5) who claimed effects of ozone on scotopic vision. We did not show such effects in these studies and the relative lack of subjective complaints from flight deck crews leads one to believe that vision is not affected operationally by 0.30 ppmv ozone.

A consideration that is central to defining the effects of ozone involves the way in which the exposure is expressed. Commonly, the so-called time-weighted average (TWA) is used to define allowable ozone exposure. The Occupational Safety and Health Administration's (OSHA) ozone standard for industrial workers is 0.10 ppmv 8 h/day for a 5-day week. However, it is clear from the literature that ozone concentration is more important than duration of exposure in producing adverse reactions. Thus, without definition of a peak limit, it would be possible for a worker to experience a symptom-producing exposure and still meet a TWA standard, provided the work environment were ozone-free for sufficient time to meet the specified TWA.

American Society for Testing and Materials recommends that 10-min sampling periods be used in defining the TWA of 0.10 ppmv. Further, ASTM recommends that 0.30 ppmv for 10 min be set as the upper limit for ozone exposure. To define TWA, ASTM uses the formula:

$$TWA = \frac{\sum C_i T_i}{\sum T_i}$$

WHERE:  $C_i$  = concentration of the  $i$ th sample.

$T_i$  = sampling time of the  $i$ th sample.

$i$  = any one sample during the time to which the TWA applies.

In our studies, ozone concentration was held relatively constant and the TWA was based on an 8-h day.

Ozone concentration should be expressed in more unequivocal terms than parts per million (or billion) by volume when the principal concern is with regard to its biological effect. Obviously, it is the number of molecules of ozone attacking cells of the respiratory passages that produces symptoms. Thus, an expression of ozone concentration by weight (mass) would accurately describe the dose, whereas the mixing ratio does not.

Part of the confusion surrounding expression of ozone dose relates to measurements techniques. Rasmussen (1) and Cook (2) have analyzed the problem of ozone measurements aloft with ground-calibrated instruments. Calibration is usually accomplished by using the ozone meter in question to measure the output of an ozone generator that has itself been factory-calibrated. However, the generator output is proportional to the amount of oxygen in the cleansed ambient air fed to it. Thus, as the generator is taken to altitude, its output drops in proportion to the decrease in partial pressure of oxygen. Under such conditions, our AID instrument shows a linear decrease in meter reading over the range of altitudes from 1,280 ft to 9,000 ft MSL. It is apparent that the actual amount of generated ozone decreases with altitude; however, the proportion of generated ozone to air should not change with altitude. This simple observation tells us that the meter does not "see" ozone in terms of parts per million, even though its meter face is so labeled.

The chemiluminescent ozone detector operates on the principle that ozone reacts with ethylene to produce light. Luminance within the reaction chamber is a measure of the amount of ozone because there is an excess of ethylene present in the chamber. Photons liberated in the reaction are transduced by a photocell into an electrical signal, which is amplified and fed to the coil of the meter galvanometer, producing a needle deflection proportional to the quantity (mass) of ozone present in the reaction chamber. Thus, the meter could as easily be made to read in mass per unit volume ( $\mu\text{g}/\text{m}^3$ ) as in ppmv.

If the meter were so calibrated,  $600 \mu\text{g}/\text{m}^3$ , for example, would be indicated as such regardless of altitude. However, when  $600 \mu\text{g}/\text{m}^3$  is translated into ppmv, altitude enters strongly into the interpretation, not because the amount of ozone changes but because the amount of air with which it is mixed changes. Six hundred micrograms per cubic meter ozone at sea level would be approximately equivalent to 0.30 ppmv. The same mass of ozone mixed with ambient air at 6,000 ft MSL would be equivalent to about 0.37 ppmv. Thus, if one had an instrument that truly measured the proportion of ozone to air and if one wanted the same amount of ozone represented by a reading of 0.30 ppmv at sea level, he/she would have to attain a meter reading of about 0.37 ppmv at 6,000 ft MSL (neglecting the effects of temperature and humidity). Obviously, a determination of ozone at altitude in terms of ppmv would represent less ozone (mass) than the same indicated ppmv at sea level.



By way of summary, it might be said that the chemiluminescent ozone detector indicates directly the biologically effective level of ozone at all altitudes without correction to sea level. It would be better, from a biological viewpoint, if such meters read directly in terms of mass per unit volume.

Folinsbee et al. (3) and Horvath et al. (4) have shown that 0.20 ppmv is the no-effect level of ozone for three levels of exercise up to 65 percent of maximal oxygen consumption. Our experiments likewise show no effect of exercise with 0.20 ppmv on objective physiological measures or on the production of symptoms. Our results are rendered conservative by the fact that our subjects breathed through a mouth-piece during the exercise episodes. Horvath has shown in his experiments that the nasal passage is a good ozone scrubber and that the threshold for effects of ozone is less when ozone is breathed by mouth than when the same amount of ozone is breathed through the nose.

Horvath has also shown that the effects of moderate exercise in an ozone atmosphere are transient and that maximal effects are shown immediately postexercise. In our experiments, full spirometry could not practically be done in the chamber immediately postexercise. However, respiratory rate and tidal volume were continuously recorded throughout the exercise periods and showed no differences between the ozone and no-ozone conditions.

Eye irritation and effects on vision are commonly cited as being caused by ozone. We saw no effects on the visual system nor were there any indications of central nervous system effects, though complaints of eye irritation (corneal/conjunctival) were associated with exposure to 0.30 ppmv ozone.

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